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THE REACTIONS OF FISH IN CONCENTRATION GRADIENTS

A COMPARATIVE STUDY BASED ON FLUVIARIUM EXPERIMENTS WITH SPECIAL REFERENCE TO OXYGEN, ACIDITY, CARBON DIOXIDE, AND SULPHITE WASTE LIQUOR (SWL)

BY

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INSTITUTE OF ZOOPHYSIOLOGY,
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SWEDEN

LUND 1961
CARL BLOMS BOKTRYCKERI A.-B.

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THESIS

BY DUE PERMISSION OF THE FACULTY OF SCIENCE OF THE UNIVERSITY OF UPPSALA TO BE PUBLICLY DISCUSSED IN THE AUDITORIUM OF THE INSTITUTE OF ZOOPHYSIOLOGY ON FEBRUARY 17, 1962 AT 10 A.M., FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

BY

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Preface and acknowledgments

This investigation was carried out at the Institute of Zoophysiology, University of Uppsala, during the years 1950—1961. There were repeated interruptions on account of other tasks, but now that I have been able to bring this work to a conclusion, it is my pleasant duty to express my gratitude to the persons who have helped me with it.

To my teacher in zoophysiology, Professor Per Eric Lindahl, the head of this institute, who first suggested the use of the fluviarium technique as a tool in evaluating some aspects of the action of water pollution upon the distribution of fish, I wish to express my sincere gratitude for his positive criticism, patient and active interest, and never-failing support throughout my work.

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Introduction and short survey of the problems

The phenomena of homing and migration of fish have been the subjects of considerable scientific interest (for literature, cf. CHIDESTER, 1924; POWERS, 1939, 1941, 1943; COLLINS, 1952, 1958; HASLER, 1954, 1956, 1960; FIELDS, 1957; BRETT and ALDERDICE, 1958; HOAR, 1958; GERKING, 1959; GUNNING, 1959). In trying to explain how fishes find their way in their natural habitats, the directive influences of environmental gradients have been suggested. Such factors as light, temperature, salinity, oxygen content, acidity, carbon dioxide tension, odorous substances, and others have been considered. The physiological background to the presumed ability of fish to discriminate between different water qualities is frequently studied, e.g. with the aid of training techniques (for references, see HERTER, 1953; and various reviews presented in BROWN, 1957).

The exploitation of rivers, inland waters, and coastal regions of the sea as receivers of waste products is a disadvantage of modern industrialized civilization which has made topical the question of how fish behave when encountering comparatively strong gradients with respect to different water qualities. A great variety of discharged substances constitutes or induces new ecological factors which may be called "artificial ecological factors" from the point of view of the original fish habitats. Within the water basins of the northern coniferous belt, sulphite waste liquor (SWL) is a frequently occurring such "artificial ecological factor". This and other waste products may also interfere with "natural environmental factors". In polluted areas O_2, hydrogen ions, and CO_2, for example, often occur, more or less locally, in concentrations which cannot be tolerated by fish.

Thus, with increasing water pollution, higher aquatic organisms, including fish faunas, gradually undergo a process of impoverishment. Discussion of the more common toxicological aspects of this urgent problem is to be found in the reviews by STEINMANN (1928), COLE (1935, 1941), ELLIS (1937, 1945), VALLIN *et al.* (1941), SOUTHGATE (1948), DOUDOROFF and KATZ (1950, 1953), RUDOLFS (1953), MEINCK, STOOF, and KOHLSCHÜTTER (1956), DOUDOROFF (1957), ALLAN, HERBERT, and ALABASTER (1958), and elsewhere. As regards injury to fish caused by SWL, references will be found in, *inter alia*, VALLIN (1935, 1939, 1958), NIGHTINGALE (1938), HALME (1950), WILLIAMS

et al. (1953), RENNERFELT (1958), PEHRSON and RENNERFELT (1958), and MOSSEWITSCH and GUSSEW (1958). On account of lack of laboratory facilities the present author was obliged to give up the original intention to make survival tests, in parallel, with the gradient experiments.

Any attempt to estimate the detrimental effects of a particular kind of pollution upon fish life involves questions, however, which cannot be answered merely by field observations or survival tests. Near the outfalls from industrial plants and municipal sewers the fish may encounter zones and strata of discharged matter which is dissolved and diluted to various extent (cf. e.g. BERGSTRÖM and VALLIN, 1937; SCHRÄDER, 1954, 1958; SALOMONSON, 1955; LJUNGGREN *et al.*, 1959). The question arises whether concentration gradients, emerging from drains containing toxic or more harmless substances, may form barriers for migrating fish. Such gradients may also restrict the distribution of more stationary species, conformably to the fact that fishes must be in physiological harmony with their habitats (COLE, 1941). Being free-swimming organisms, they may be able or not be able to avoid locally unfavourable conditions. Apart from possible toxic effects, SWL, for example, may act as a directive stimulus *per se* or by secondarily induced changes in the original environments of the fish. But, on the whole, the ability of various species of fish to avoid contacts with poisonous or noxious substances has not been sufficiently investigated.

Before anything can be said about the directive influence of a particular factor in nature, knowledge of the preference reactions in well-defined gradient experiments must be gained (cf. SHELFORD and ALLEE, 1913, p. 316 ff.; BÜCKMAN, 1956; and others). The experimental approach to these problems is still very incomplete, though a number of devices have been developed (cf. Chapter I).

The fluviarium technique was first described by HÖGLUND (1953) and later revised by LINDAHL and MARCSTRÖM (1958). Using this technique, the main purpose of the present study is to gain more evidence of how fish behave when meeting different water qualities. The experiments were planned to investigate possible physiological mechanisms and reaction patterns that may make it possible for fish to find their way out from and avoid adverse concentrations in various types of graded environments. Unconditioned species representing different physiological and ecological types (hardier and more sensitive ones) have therefore been studied on a comparative basis in similarly arranged series of steeper and steeper gradients established perpendicularly to the direction of flow through the fluviarium. The gradients usually start from nil, that is, almost pure water is found along one side of the test yard. The most commonly used gradients (α_1-gradients, cf. Fig. 1) consist of ten concentration steps and rise to the highest concentration along

the opposite side. The steepest α_1-gradients of most experimental series usually extend well beyond the actual maximum tolerable concentrations. The test agents are chosen as representatives of both "natural" (O_2, pH, and CO_2) and of "artificial ecological factors" (SWL).

The perceptual background to the preference reactions is studied by comparing the reactions before and after the elimination of particular sense organs. The significance of the reactions will be briefly discussed according to general biological principles put forward by, e.g. ODUM (1954), TINBERGEN (1955), and WOODBURY (1956). From an evolutionary point of view it is interesting to find out to what extent fish posess mechanisms and exhibit reactions that protect them against adverse influences from the graded environments. Essential differences might be expected as regards the reactions to "natural" and "artificial ecological factors". The animals are physiologically adapted by means of natural selection to withstand or escape only the first-mentioned kind of environmental factors.

The present study has been performed exclusively in the laboratory. All quantitative information arrived at is based on film records of the momentary positions of fish in pure water (control conditions) and in stable gradients of various steepnesses. The preference reactions were obtained in a confined space (23.5×33 cm) of the streaming aquarium. Objections can always be raised against an experimental analysis of the present kind and the results must be estimated against the background of the technique employed. An attempt is made in the present study to survey, as completely as is reasonable, various methodological problems which may be of importance for the evaluation of the reactions obtained.

It must also be emphasized that preference reactions obtained with steep artificial gradients are not necessarily applicable to similar conditions in the receiving basins for waste effluents or to less pronounced gradients in more uncontaminated waters. This has already been pointed out by DOUDOROFF (1938, 1957) and by DOUDOROFF and KATZ (1950, p. 1433). In nature the causalities are furthermore complicated by the existence of biotic as well as abiotic ecological factors, which may be counteractive or synergistic in their action upon the dispersal of fish. Moderate pollution often contributes to increase the productivity of a water. Changes in he abundance of food and competitors may attract certain fish (cf. METCALF, 1942). But a strongly increased production, especially when followed by less favourable changes in the abiotic environment, may make it impossible for more or less sensitive forms to live in aeras contaminated more severely (cf. e.g. SAHA, SEN, MUKHERJEE, and CHAKRAVARTY, 1958).

However, drawbacks of a more restricted methodological kind are also attached to the present results. Subtle reactions which may occur in nature

may be suppressed under the artificial conditions employed. The physiological status (endocrine cycle, vitality and common well-being, conditioning, training, and so on) is not easy to control. Such difficulties do not necessarily invalidate conclusions, however, which are based on a comparative analysis of reproducible reactions of different species tested under well-defined and highly standardized gradient conditions.

Chapter I

Material and methods

I. Animal material

All the fish materials chosen for the present study are freshwater species, coming from Swedish natural waters and rearing plants. The wild fish are collected in traps or in scap-nets. Thus damage to the fish in the process of capture is prevented as far as possible. The fish are caught on several occasions in the river Fyris or in the lake Mälaren in the same drainage system, in the river Dalälven at Älvkarleby, near the shore of two shallow creeks of the Baltic at the border of the Bothnian Sea at Billhamn and at Ängskär, and in the river Indalsälven at Hölle. The reared fish come from troughs and a natural rearing pond, viz. Hyttödammen, at the Fish Rearing Plant at Älvkarleby on the river Dalälven; from the rearing ponds, Harviksdammarna, at Dannemora, within the drainage system of the river Fyris; from rearing ponds at the Fish Hatching Plant at Aneboda in the province of Småland; from troughs at the Salmon Research Laboratory and Fish Hatching Plant at Hölle and, finally, from Bonäshamn situated at the same drainage system.

In the laboratory the fish are kept in tanks supplied with running water. The test fish are acclimatized to the same water quality conditions that prevail in the test yard of the fluviarium during control periods. It was found impossible to avoid losing amongst the more sensitive species such as the salmonids during the first few days of storage after the transport to Uppsala (cf. e.g. HART, 1952, p. 5). No experiments were performed until the stored population seemed to be acclimatized.

The fish are fed with liver sausage, and generally they feed well during storage. After the first acclimation period in the tanks they seem to thrive well, and mortality or any signs of disease such as *Saprolegnia* are practically non-existant over a period of many years. When any visible signs of decreased vitality are noticeable the population is discarded.

Only small specimens, i.e. in most cases juvenile ones, can be studied in the confined space of the test yard of the streaming aquarium. The characteristics of the specimens used as regards species, length, and weight, etc., are given in connection with the presentation of the particular experiments in

Table 1. The specimens are weighed alive. The length of the fish is measured as "total" length with the tail in a normal position. The length of crayfish is given as the distal length between rostrum and telson. The taxonomy used refer to NYBELIN (1956) and in the case of *Coregonus* to SVÄRDSON (1957) and *Astacus* to Dr Å. HOLM (oral communication).

II. The technique

A. Earlier techniques

The direct effects of differences in water quality on the behaviour of fishes have been studied experimentally mainly along the following lines. (1) The *gradient tank method* was first introduced by SHELFORD & ALLEE (1913) and is described in detail by SHELFORD (1939). Pure water and a watery solution of the agent to be tested are supplied to either end of the tank and drained to a common outlet in the middle. Thus a gradient, though not very well defined, is established in the region of the tank where the two kinds of fluids meet. This method is later used in many investigations, e.g. by WELLS (1915 a, b, 1918), SHELFORD and POWERS (1913, 1915), SHELFORD (1917, 1918), POWERS (1921), HALL (1925), POWERS and CLARK (1943) and in a modified way by JONES, WARREN, BOND and DOUDOROFF (1956). (2) Adopting the same principle JONES (1947, 1948) used a horizontal glass tube with a capacity of about 400 ml for establishing a sharp border between pure water and a definite concentration of a given test substance. A similar *choice apparatus* of about the same dimensions was earlier used by OLTHOF (1941) in his rather undefined studies on *i.a.* the preferences at 22—23° C of *Lebistes* which was encountered with different oxygen and carbon dioxide concentrations. A modification of JONES' testing tube was also used by HODGSON (1951) studying the stimulation of *i.a.* ions on an aquatic beetle. (3) Studying the reactions of *Girella nigricans* to horizontal temperature gradients DOUDOROFF (1938) worked with a *compartmented tank* provided with a number of separate inlets and outlets to each compartment. Another type of apparatus for the study of temperature selection of fish in horizontal gradients was used by SULLIVAN and FISHER (1952, 1954). A compartmented sharp-gradient tank was used by BAGGERMAN (1957) studying the reactions of *Gasterosteus aculeatus* to fresh and salt water. In a somewhat modified apparatus the responses of juvenile salmon (*Onchorhyncus spp.*) to sea water were studied by HOUSTON (1957). (4) BRETT (1952) devised a *preferred-temperature tank* with a vertical gradient. This is employed also by PITT, GARSIDE, & HEPBURN (1956). (5) CHIDESTER (1920) made some field observations supplemented by an experimental study (CHIDESTER, 1922) in which fish in one trough were met by water of different salinities coming from two other troughs. A similar, four-armed apparatus was used by WISBY and

Table 1. Animal material and experiments.

Test animals							Experiments			
Family and species	Number	Weight in g Range and mean	Length in mm Range and mean	Perceptual state	Origin	Age	Date	Test agent	Feed-water	Reference to figs., tables, or pages [1]
Percidae										
Perca fluviatilis L., perch	7	—	About 50	intact	Billhamn	yearlings	25/11, 1957	SWL	Uppsala	18, 48
Gasterosteidae										
Gasterosteus aculeatus L., three-spined stickleback	10	—	—	»	»	adults	24—26/9, 1958	SWL	»	51
	10	1.1—2.5, 1.9	48—64, 58	»	»	»	18—26/11, 1958	HCl	»	13, 14, 36
	10	—	—	»	Ängskär	»	19/5, 1959	SWL	»	51
Cyprinidae										
Tinca tinca (L.), tench	5	9.5—22.5, 16.6	90—113, 108	»	Aneboda	hatched in June -57	17/11—30/12, 1959	HCl	»	36
Phoxinus phoxinus (L.), minnov	3	—	—	»	Billhamn	adults	26/3, 1957	SWL	»	39
	3	—	77—90, 83	»	»	»	6/9—16/11, 1957	pure water	»	8
	1	—	—	»	»	»	3—9/5, 1958	SWL	»	39
	1—3	—	—	»	Hölle	»	30/9—10/12, 1958	SWL	»	7, 39, 40, 41
	8	—	—	»	»	»	10—11/11, 1958	SWL	»	39
	5	—	—	»	»	»	26—28/11, 1958	HCL	»	36
	3	—	—	intact and olfactory organs eliminated	»	»	26/1—2/2, 1959	SWL	»	39, table 15
	4	4.2—6.2, 5.2	78—86, 82	lateral organs eliminated	»	»	5/8, 1960	HCl	»	15
Leuciscus rutilus (L.), roach	10	0.9—2.3, 1.5	45—60, 52	intact	Älvkarleby	—	6/11—20/12, 1958	pure water, SWL, etc	»	10, 11, 19, 20, 21, 22, 23, 42, 45, tables 8, 9, 10, 11, 12
	5—7	0.8—1.8, 1.4	48—62, 57	intact and olfactory organs eliminated	»	—	19/1—25/2, 1959	current, HCl, SWL	»	12, 19, 20, 29, 31, 36, 43, 46, table 11
	9	7.2—11.0, 9.6	97—105, 103	intact	Lake Mälaren	—	12—16/5, 1959	SWL	»	[2]
	10	6.9—10.6, 8.6	90—110, 96	»	»	—	6/5—10/6, 1959	O_2	»	17, 25, 26
	5	5.5—9.8, 7.4	94—105, 100	»	»	—	15—17/6. 1959	HCl, NaOH, NaCl	»	16, 29
	10	2.4—4.4, 3.0	64—81, 70	»	Hölle	—	22—27/7, 1959	HCl	Hölle	32, 37
	10	2.9—6.7, 4.8	69—91, 81	»	»	—	2—4/8, 1959	light, SWL	»	44, p.
	5	2.7—5.7, 4.5	69—89, 80	lateral organs eliminated	»	—	4/8, 1960	current, HCl	Uppsala	15, 19
Leuciscus idbarus (L.), ide	7	5.8—13.1, 9.0	85—125, 103	intact	Billhamn	—	28/11 1957—21/5, 1958	pure water, SWL	»	9, 47
Esocidae										
Esox lucius (L.), pike	5	4.4—7.0, 5.16	92—107, 95	»	Aneboda	hatched in May -58	15—16/7, 1958	SWL	»	p. 128
	9	—	—	»	»	»	4—5/8, 1959	SWL	»	p. 128
Salmonidae										
	10	3.17—6.40, 4.39	67—88, 76	»	Älvkarleby	yearlings	20—22/10, 1958	SWL	Uppsala	50
	10	3.0—5.7, 4.1	77—90, 82	»	»	»	21—29/4, 1959	SWL	»	50
	10	2.9—6.3. 4.0	75—95, 83	»	»	»	23—29/4, 1959	HCl	»	33, 36, 38
Salmo salar L., Atlantic salmon	10	—	—	»	»	»	8—9/5, 1959	O_2	»	27
	10	2.9—6.6, 4.5	67—92, 79	»	Hölle	hatched in April -58	28—29/7, 1959	HCl	Hölle	30, 34, 37
	10	1.2—1.5, 1.3	44—62, 54	»	»	hatched in April -59	30/7, 1959	HCl	»	table 13, p. 99
	10	— , 3.9	— , 82	»	Älvkarleby	»	4/11, 1959	O_2	Uppsala	27
	4	2.6—7.5, 5.6	83—102, 94	lateral organs eliminated	»	»	3/8, 1960	HCl	»	15
Salmo trutta (L.), brown trout	10	4.8—10.6, 7.3	75—102, 87	intact	Aneboda	hatched in April -58	24/11—1/12, 1958	HCl, SWL	»	36, 50
Salmo alpinus (L.), char	5	4.6—11.2, 7.9	80—115. 94	»	Bonäshamn	»	30—31/1, 1959	HCl, SWL	»	36, 50
Salmo fontinalis Mitchill, brook trout	10	1.7—6.3, 4.07	60—90, 77	»	Aneboda	»	2—9/12, 1958	HCl, SWL	»	36, 50
Coregonidae										
Coregonus nasus Pallas, whitefish	4	—	—	»	Älvkarleby	yearlings	8/11, 1957	SWL	»	49
	2	—	—	»	»	»	22—29/4, 1958	SWL	»	49
Crustacea										
Astacus astacus L., crayfish	5	11.3—20.8, 17.0	62—82, 73	»	Dannemora	—	11/5, 1959	O_2	»	28

[1] Unless otherwise stated, the figures given refer to illustrations.

[2] A reproduction of the results presented in Fig. 42 (cf. p. 116).

Hasler (1952) to test the reactions of unconditioned salmon to various odours (Hasler, 1957). (6) Besides the modified gradient tank, Jones, Warren, Bond, and Doudoroff (1956) in their studies on the reactions of juvenile salmonids to pulp-mill wastes, used an *avoidance tank* partioned at one end into four parallel channels. A slight modification of this was used by Whitmore, Warren, and Doudoroff (1960) in a study of the reactions of salmonid and centrarchid fishes to low oxygen concentrations. (7) Collins (1952) constructed an experimental trough similar to the last mentioned type of avoidance tank. It was used partly submerged into the stream in the direction of the water flow. Open at either end and subdivided in the upper third into two uniform channels it conveyed water of two different qualities. Alewife (*Pomolobus pseudoharengus*) and glut herring (*Pomolobus aestivalis*), when progressing upstream during the spawning run, were directed with screens into the trough. At the entrance of the trough each fish was subjected individually to a mixture of the unadulterated and modified water. Thus, starting in the middle of a steep gradient they had to choose between two water qualities. An apparatus of this type was also used by Smith and Saalfeld (1955) studying the aversion of *Thaleichtys pacificus* to certain industrial effluents. Focusing the problem of guiding downstream migrant salmon young Brett, MacKinnon, and Alderdice (1954) used a large experimental trough similar in construction and submerged directly into a stream. See also Brett and Alderdice (1956, 1958) and Fields (1957).

B. **The fluviarium technique**

The *fluviarium method* for studying the reactions of fishes in concentration gradients of chemical and other agents was first described by Höglund (1953). Lindahl and Marcström (1958) introduced essential technical improvements for the stabilization of the flow which are adopted in the present model. They constructed two jet damping obstruction boxes, used also as mixers, and adherent funnel-shaped mouthpieces conveying water and test solution into the proper experimental trough. The earlier preliminary report by the present author was written in conformity with outlines suggested by Professor P. E. Lindahl at the request of Dr. A. Lindroth.

In the fluviarium technique ten well defined concentration steps are arranged perpendicular to the flow in an experimental flume. The type and angle of the gradients can be altered as desired according to the problem that is to be studied experimentally. The flume is covered at its upper end and open downstream. Ten longitudinal sections are arranged in the middle part, i.e. in front of the proper test yard. In the last one test fishes are allowed to swim about freely, choosing positions in the graded environment obtained. When the rate of flow is held within certain limits the water flows through the open parts of the apparatus without vortices. Then the concentration

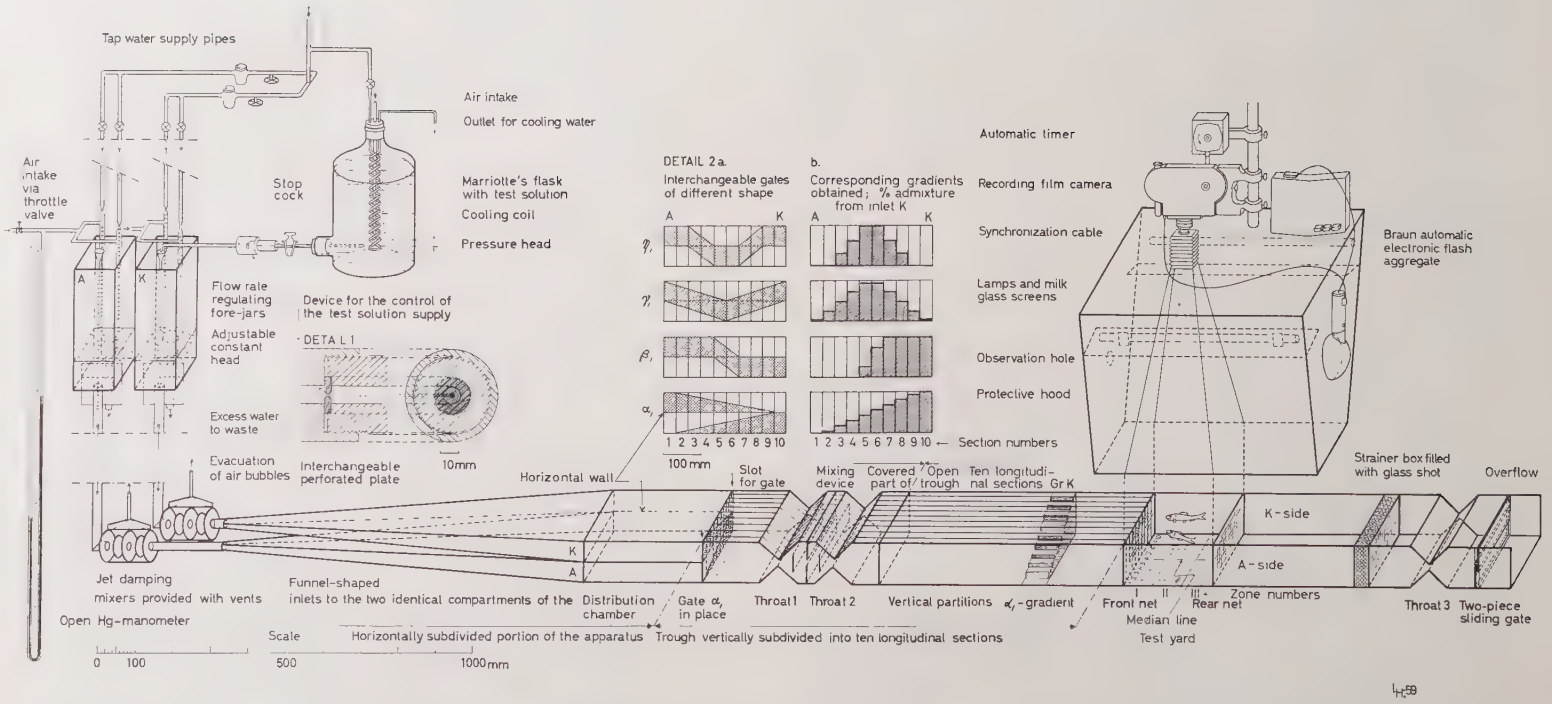


Fig. 1. General view of the fluviarium. Fore-jars and experimental trough are presented as the limiting lines of the enclosed water mass. Only one manometer is shown.

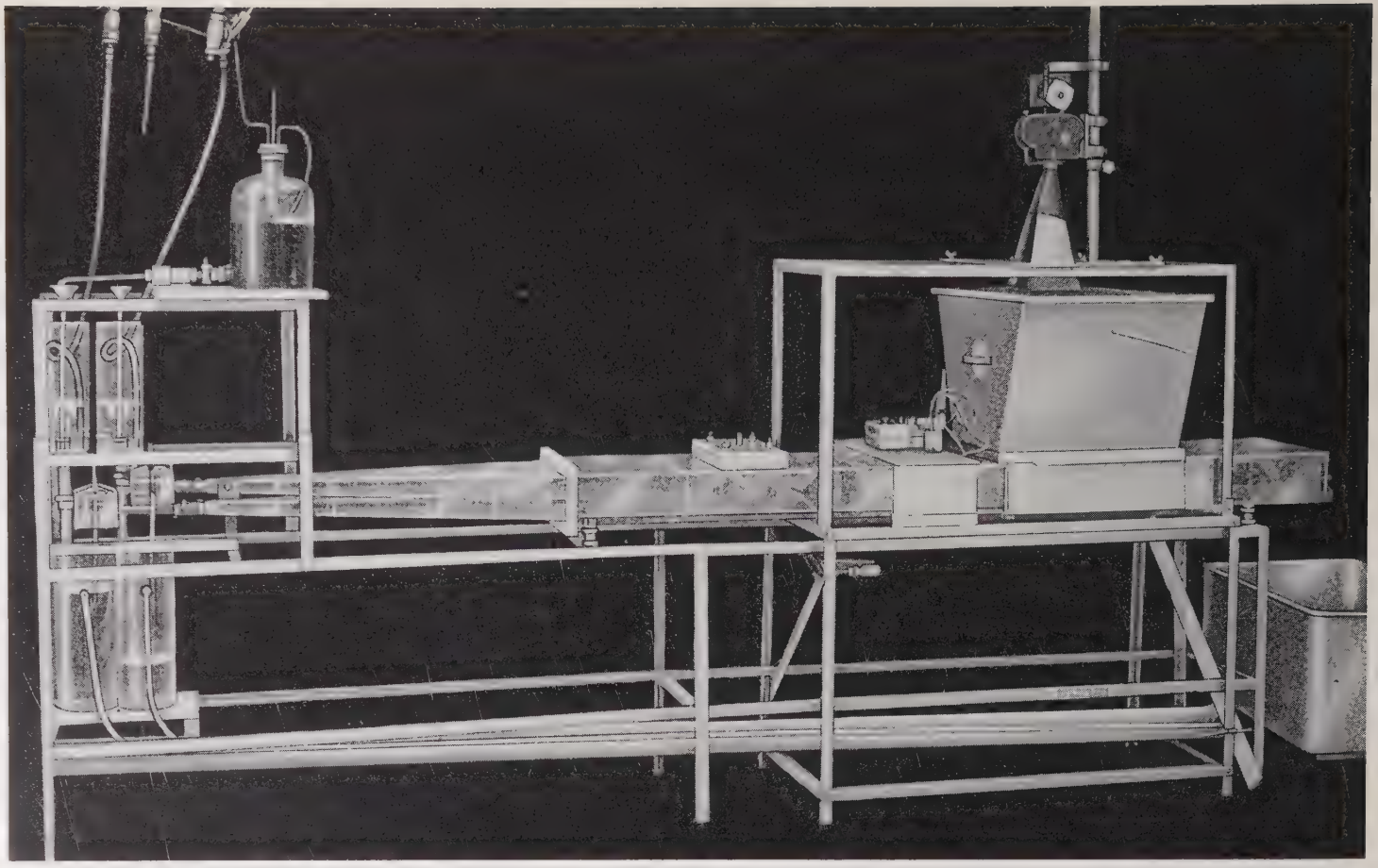


Fig. 2. Photograph of the fluviarium. The box superposed on the covered part of the experimental trough was constructed for the evacuation of air from the distribution chamber and throated mixing device in this part of the flume.

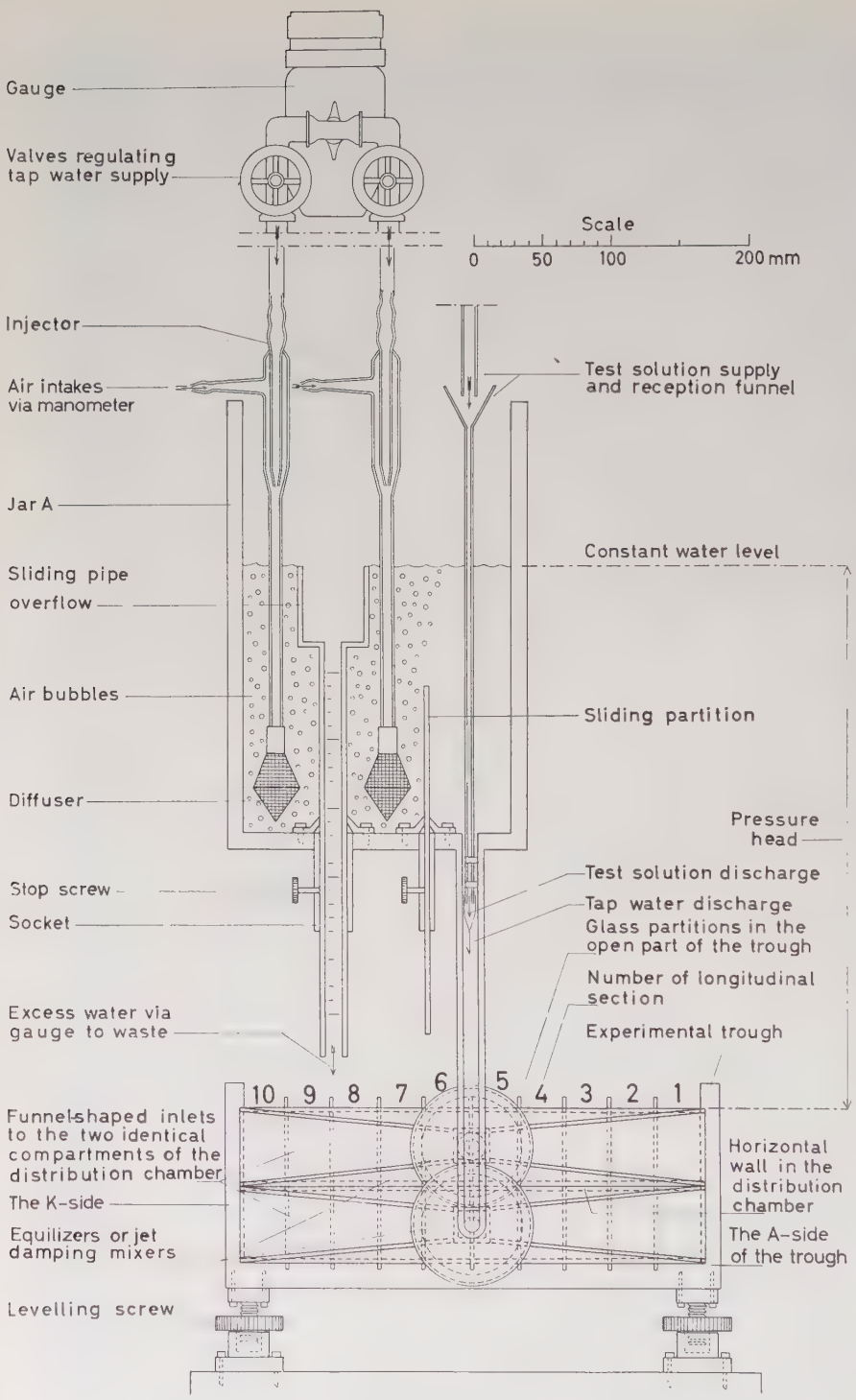


Fig. 3. Scheme of the fluviarium as seen from its upstream end.

gradient once established in the upper covered part of the trough will change comparatively little during the passage through the test yard, which takes less than 30 seconds at 1 cm/sec which is the rate of flow most generally used in the present experiments. As the gradient is continously rebuilt at the front net of the yard, disturbances of the gradient caused by the movements of the fishes do not considerably affect the results.

C. Description of the modified fluviarium used in the present study

The apparatus was further modified in many respects and entirely rebuilt for the present purpose *inter alia* in order to use dissolved gases as test agents. For this reason a new description will be given here with reference to the earlier descriptions mentioned. The present construction is shown in Fig. 1—3, which are largely self-explanatory.

The most essential part of the present apparatus is built of transparent polymethacrylate plastic, called Bonoplex, manufactured by AB Bofors Nobelkrut, Bonoplexfabriken, Tidaholm, Sweden. If a coloured component is used as a model substance this permits the observation of the flow of water in all parts of the apparatus. Another advantage of the plastic material is its freedom from corrosion within wide limits. During work with acid solutions no ions are dissolved from the material. To what extent the plastic takes up odourous substances has not been thoroughly studied. The present results do not indicate any disturbances of this kind.

The main constituents of the present apparatus are as follows:

(1). *Water and air supply*

A. Tap water supply pipes, regulating valves, and gauges.

B. Two air injectors provided with diffusers in each fore-jar. Two open Hg-manometers, each connected to the two injectors of one fore-jar.

(2). *Arrangements for the control of water supply*

A. Two constant-level fore-jars, designated A and K.

B. Arrangements for accurate adjustment of the pressure head in each fore-jar by means of vertical sliding pipes and drains.

C. A number of equalizers constructed as obstruction boxes, throats, nets, and strainers for the stabilization of the flow.

D. A two-piece sliding weir regulating the overflow at the rear end of the flume.

(3). *System for air evacuation and air bubble trapping*

(4). *Arrangements for the control of test solution supply*

A. A Marriotte's flask provided with a cooling coil.

B. A stop-cock and a control device at the outlet of Marriotte's flask, i.e. a plastic box provided with two interchangeable concentrically bored screw-lids. The latter fix one from a series of eleven constriction plates with differently sized central apertures (Fig. 1, detail 1, and Fig. 5). Reception funnels and central tubes convey test solution into the mixers also functionating as equalizers (cf. (5). B. and Fig. 3).

(5). *Experimental trough with auxiliary gears*

A. Two identically funnel-shaped inlets open into the proper experimental trough which in its upstream part is subdivided by a horizontal wall into two identically shaped tunnels with rectangular sections.

B. One water jet damping obstruction box or equalizer at the entrance to each of the two funnelled inlets to the trough. It likewise serves as a mixer of undiluted test solution and pure tap water.

C. The proper trough supported by seven levelling screws.

Trough closed.	a. A distribution chamber with detachable gradient-generating obstruction gates provided with slanting apertures of different shapes. (Fig. 1, detail 2).	Trough subdivided by a horizontal wall.
	b. A detachable mixing device consisting of ten identically throated tunnels.	Trough subdivided by vertical walls into ten longitudinal sections.
Trough open.	c. Ten identical open channels in the middle part of the trough constituting the extensions of the ten tunnels mentioned under (5). C. b.	
	d. A test yard.	
	e. A strainer box filled with glass beads and a throat constriction for the stabilization of the flow in the rear part of the open trough.	Trough not subdivided.

E. Sheltering hood provided with two symmetrically arranged tubular lamps and opalescent glass screens for the indirect illumination of the test yard.

(6). *Arrangements for the recording of the momentary positions of test animals*

A. Film camera.

B. Automatic release.

a. Synchronous electric motor.

b. Interchangeable gear-wheels producing accurate intervals of different lengths between exposures (15, 30, or 60 seconds).

C. Arrangements for the darkening of the test yard.

D. Electronic flash aggregate.

D. A closer approach to the function of the fluviarium

In order to facilitate the creation of reproducible and stable gradients of a special kind the necessary procedures have been standardized as far as possible. Great care was taken to apply automatic devices. After calibration of the apparatus any desired experimental condition could then conveniently be reproduced by simple alterations in the standardizing devices.

1. *Water supply and aeration*

Tap water is supplied to each of two equal flow rate regulating fore-jars measuring 12×23×35 cm. As Uppsala tap water contains practically no dissolved oxygen (cf. p. 32) the supply water has to be oxygenated. This is performed in each fore-jar by the aid of two coupled injectors which are connected to a common open Hg-manometer which is used to control the amount of injected air. The air stream is regulated so as to produce an oxygen saturation in the test yard of c. 75 per cent at the ordinary flow rate (9° C, 1 cm/sec) of the water, which is controlled by ordinary Winkler analyses. Higher values of oxygen saturation are obtained with difficulty, possibly on account of the liberation of some dissolved air at constrictions in the covered part of the apparatus. This is in accordance with the lowering of the pressure following Bernoulli's law.

The diffusers, shown in Fig. 3, exert a damping influence on the kinetic energy of the emerging water jets. For the same reason they open well beneath the water level of the jar, since the surface must be kept as smooth as possible in order to obtain a constant head. A still more perfect performance might be obtained if the diffusers were submerged in a jet damping pouch with elastic walls or some similar partition construction below the floor of the jars.

Importance is attached to the practical difficulty of obtaining a homogeneous water mass flowing through the covered parts of the apparatus. Air has to be evacuated from pockets and corners under the covering roofs, when the apparatus is filled with water. At the upper part of the mixing boxes and in the roof of the throat constriction box, air vents are pierced in the shape of slots which are provided with well fitting plugs. Air bubbles are prevented from being sucked from the fore-jars into adjacent covered parts of the apparatus by a sliding partition (Fig. 3). In accordance with Bernoulli's principle the liberation of minute air bubbles could not be altogether prevented. Small amounts of air gather beneath the covering roofs during the course of an experiment. This effect is, however, limited to the funnelshaped inlets of the trough, and does not affect the experimental conditions in the test yard to any appreciable extent.

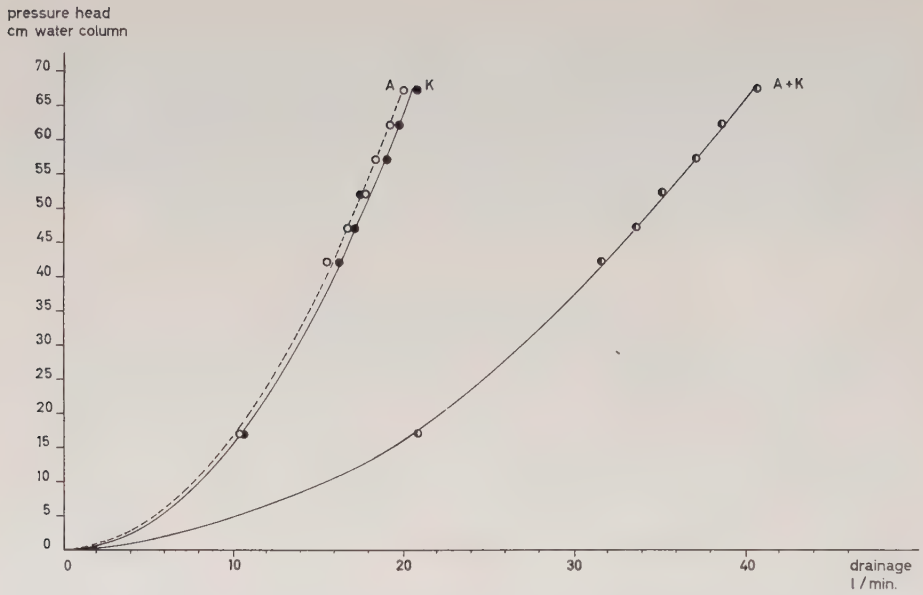


Fig. 4. Pressure head in the fore-jars in relation to drainage through the experimental flume. A and K denote the flow from the respective fore-jars; A+K the total one.

2. *Arrangements for obtaining a constant flow*

After the publication of the earlier descriptions the apparatus has been modified, with the aim of obtaining greater accuracy and constancy of pressure heads for regulating the discharge of feed water and test solution. The pressure head is measured as the vertical distance between the water levels in the fore-jars and the test yard. The concentration level of any gradient in the latter depends on the amount of test solution of a definite concentration supplied in relaion to the amount of pure water supplied (cf. HÖGLUND, 1953, p. 257). According to Torricelli's theorem (cf. p. 21) a constant pressure head results in a constant drainage. The water surfaces in the fore-jars are kept at the same level and thus the water masses act as constant heads. In the present construction the accurate adjustment of the head of water is obtained by an open standpipe with an enlarged cylindrical upper mouth. It functions as an overflow drain. The standpipe can be slid vertically, and is guided by a sleeve. Leakage is avoided by a tight closing ring of polyethene. By means of a stop screw in the sleeve the overflow device can be fixed in the chosen position. The volume of test solution added to one of the fore-jars amounts, at most, to one per mille of the total flow. This can be disregarded in the discussion of the relationship between head and flow through the apparatus. In order to study this relationship the excess water from each fore-jar and the total flow through

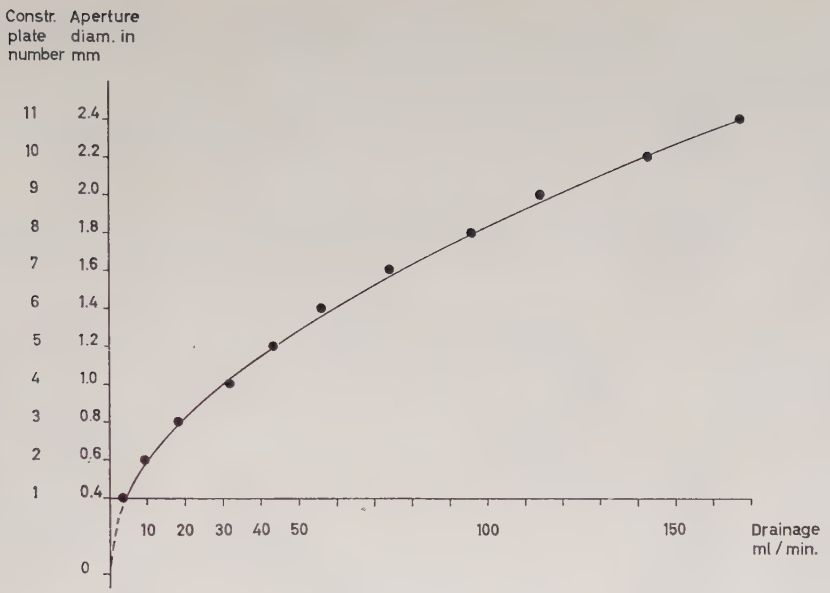


Fig. 5. Aperture diameter of constriction plates in relation to the outflow from Marriotte's flask of tap water at 10°C.

the trough are measured by collecting the discharge in the pertinent volumetric vessels (Fig. 2), while noting the times with a stop-watch. The corresponding water flow is calculated. The relationship between pressure head and discharge, seemingly parabolic, is shown in Fig. 4.

3. *The maintenance of a constant supply of test solution*

A 10 l flask containing concentrated test solution is constructed according to Marriotte's principle. Thus the discharge through the outlet tube will be the same, independent of the height of the column of fluid in the flask, and therefore depending exclusively upon the width of the outlet orifice following the standard formula (Torricelli's theorem) relating to circular free-outflow. The latter is controlled by a simple device made of Bonoplex plastic (Fig. 1). Eleven constriction plates with central orifices of different diameters (0.4—2.4 mm) were turned from the plastic material. Any one of these can be inserted into a circular holder with an interchangeable screw-lid. Two different lids are used, the only difference between them being the nozzles, 10 or 25 cm long, fitting the distance from the control box to the reception funnel of fore-jar A or K respectively.

Before being sucked into the outlet tube regulating the discharge the test solution contained in the flask has to pass a fine meshed filter of sufficiently high penetration capacity. The relation between the diameter of orifice and the discharge of test solution is calibrated with the series of constriction

plates. The results obtained with tap water is shown in Fig. 5. As might be expected a seemingly parabolic curve is obtained.

For the maintenance of a constant head of the test solution in the Marriotte's flask the temperature must be constant during the course of an experiment.

Furthermore temperature differences must not occur across the width of the trough because BULL (1928, 1936, 1957) and DIJKGRAAF (1940) have shown that fishes are able to form conditioned responses to very small temperature changes. Preference reactions of fish in temperature gradients are also reported (cf. SHELFORD and POWERS, 1915; DOUDOROFF, 1938; FISHER and ELSON, 1950; BRETT, 1952; SULLIVAN and FISHER, 1953, 1954; PITT, GARSIDE, and HEPBURN, 1956).

For these reasons the difference in temperature between the two fluids to be mixed, viz. test solution and feed water, should be as small as possible. The largest aperture (diameter 2.4 mm) used in the present investigation causes a discharge of 170 cm^3/min from the Marriotte's flask. With the use of this plate and gate α_1 a 10° test solution supplied to 9° feed water flowing through the apparatus at the drainage of about 20 l/min (about 1 cm/sec) will produce a rise of temperature of about 0.02° C at one side of the test yard. At lower rates of water-flow the use of constriction plates with smaller apertures is therefore recommended. In these cases the steepness of gradients can be regulated by altering the concentration of the test solution.

Before the start of an experiment the test solution is cooled to 9° C. During the course of experiments the temperature is kept constantly near that of the feed-water by the aid of a double cooling coil of 6 mm glass tubing, supplied with circulating tap water and inserted in Marriotte's flask (Fig.1).

4. *The first step of continuous mixing of original test solution with pure feed-water*

The cylindrical constriction boxes (Fig 1) cause an even spreading of the kinetic energy of the water flowing into the experimental trough. They also function as mixers. Seen from the centrally placed circular opening in the foremost wall the water jet from one fore-jar is first of all stopped by a centrally placed circular plate. It has to pass this obstruction peripherally as a sheet along the cylindrical wall of the box only to be deflected once more by a peripheral ring-shaped diaphragm. After a third obstruction of the same kind as the first one the water passes the centrally placed outlet tube in the back wall. This tube projects 10 mm into the box.

As can be seen in Fig. 3 concentrated test solution introduced into the open reception funnel of the one fore-jar flows through a narrow tube (inner diameter=8 mm) which is placed concentrically within the wider outlet tube (inner diameter=25 mm) conveying tap water from the fore-jar. It

projects more than 10 cm into the upper part of the latter in order to prevent the loss of any test solution with the excess water.

During the passage of test solution and water through the equalizing box, the obstacles causing heavy turbulences bring about a complete mixing of the two liquids. The homogeneously mixed solution of diluted test substance flows into one of the mouth-pieces and further into the distribution chamber. Simultaneously pure tap water enters through the other inlet.

5. *The proper experimental trough*

All auxiliary gears put into the flume are removable. The inner surface of the bottom and the walls is smooth without any obstacles which might give rise to an uneven flow, or cause disturbing eddies.

The trough is supported, and can be accurately levelled by a system of seven levelling screws. The inner dimensions corresponding to the water body filling up the apparatus is shown in Fig. 1, the thickness of the plastic material in Fig. 3.

In the place, where the covered part of the trough joins the open part the free level of the water is kept at 11 cm above the floor, i.e. not lower than in the covered part. This is achieved, irrespective of the rate of flow, by adjusting the sliding gate at the rear end of the open trough. The flow through the open part is again equalized by a strainer box situated behind the test yard. It is made of stainless steel gauze (meshes 1 mm^2) and is filled with glass beads (diameter c. 5 mm). It causes a drop of level of c. 10 mm. In order to prevent a vertical velocity gradient from being induced by faster flowing surface layers, the liquid is forced to pass a third throat (cf. Fig. 1) before reaching the overflow weir.

6. *The state of flow in the open part of the trough*

Reynold's number (R_n)

$$R_n = \frac{v \cdot d \cdot p}{\mu};$$

can be used in order to describe the state of flow through the open trough.

Inserting the relevant values, i.e. p (density) ≈ 1 g/cm^3, μ (viscosity at 9° C) $= 1.3462 \times 10^{-2}$ poise according to HODGMAN (1952, p. 1882), d (smallest dimension of cross-section[1]) $= 3.1$ cm, and v (velocity of flow) ≈ 1cm/sec, into this expression, we get $R_n \approx 230$. If Reynold's number is below about 2000, the flow will be viscous (ADDISON 1949, p. 311). Considering this we find the flow of the water in the longitudinal sections to be well below the critical value mentioned. Consequently the water will move in smooth,

[1] The estimate is made for one longitudinal section the breadth of which is 3.1 cm.

regular, and parallel paths. This presumably is a necessary condition for the establishment of stable gradients which will change very little when passing through the test yard. It furthermore contributes to a minimal gas exchange between the watery phase and the air during the passage of the fluid through the open part of the apparatus (cf. Chapter II).

7. *The generation of gradients*

The generation of different kinds of gradients was described in detail by HÖGLUND (1953). The two kinds of fluids supplied through the funnel-shaped mouthpieces of the experimental trough are distributed over the width of the trough in mutually inverse quantities. By means of gates of different shapes different proportions of diluted test solution and pure water are conveyed into the ten longitudinal tunnels of the middle part of the trough (see Fig 1, detail 2).

By combination of a few standard conditions a great variety of well defined gradients can be obtained. The following factors can be varied, i.e. (1) the *type of gradient generating gate* (α, β, γ or η, cf. Fig. 1, detail 2), (2) the *pressure head of flowing water* (Fig. 4), (3) the *aperture of the constriction plate* for test solution supply (Fig. 5), (4) the *concentration of test solution,* and (5) the *choice of test solution supply to one of the fore-jars A or K* (Fig. 1).

8. *The second step of steady mixing of diluted test solution with pure feed-water*

The mixing procedure by aeration in the subdivided middle part of the trough employed by LINDAHL and MARCSTRÖM (1958) involves the risk of some indefinite elimination of dissolved gases and volatile substances from the flowing liquid. This is avoided here by using symmetrically arranged constrictions in the distribution chamber which in the rear part is subdivided by means of vertical walls into ten equal tunnels.

By means of throats and constriction plates bringing about heavy vertical turbulences in a similar way as in the equalizers at the trough inlets a homogenous solution is obtained in each passage. After the passage of this throated part, which constitutes a second mixing step of the apparatus, a concentration gradient is obtained over the width of the trough. During the flow in the ten open channels down to the front net of the test yard the different mixtures generated as described above are still kept separate by vertical glass sheets.

It is a disadvantage that at very low flow rates heavy solutions of not readily soluble substances may tend to remain stratified at lower flow rates, i.e. pass along the bottom surface.

9. *The test yard*

The test yard is confined by the lateral walls of the trough and the two nets of stainless steel of the same kind as that in the strainer box. It measures 23.5×33 cm. A removable white painted aluminium sheet is inserted close to the underside of the transparent bottom. In order to prevent disturbances from outside, two other sheets of the same material are placed outside the lateral walls of the yard. Similarly an illumination hood gives protection from disturbing visionary impression from above. The sheets as well as the interior of the hood are painted white. A camera and other attachments are fixed on stands separated from that of the trough proper. They rest on a foundation which eliminates any vibrations from the surrounding laboratory.

Two symmetrically placed 40 W tubular incandescent bulbs (type Linestra, manufactured by Philips, Holland) screened by sheets of opalescent glass provide an evenly dispersed illumination of the test yard. This illumination is sufficient for exposures with a film camera.

In an attempt to produce an even distribution of temperature, flow velocity, oxygen content, and light the highest degree of symmetry is sought in every possible way.

The positions taken by fishes in the test yard are conveniently described by referring to the hypothetical division shown in Fig. 1. In the flow direction the yard is thought to be divided into three equal zones which are designated I, II, and III. Transversally it is thought to be divided into ten equally sized sections, 1—10, corresponding to the longitudinal sections of the middle part of the trough. In Fig. 1 also an imaginary median line along the long axis of the trough is indicated. Actually only four dark parallel lines corresponding to the borders of each double-section are painted on the bottom sheet (cf. Fig. 7). The quantitative results arrived at are mainly presented graphically. The shape and the hypothetical division of the test yard are simply recognized in the various types of graphs (cf. p. 49 ff.).

10. *The designations of gradients*

The gradient generating gates are consistently placed in the position shown in Fig. 1. Here, see detail 2, the gates are drawn, as seen, against the direction of flow. Consequently two kinds of gradients — inversed in relation to each other — can be obtained by switching the test solution supply from one fore-jar to the other, while keeping all other conditions unchanged. Such gradients, the forms of which might be expected to be mirror images of each other, are called *corresponding inversed gradients.*

One side of the trough is called the *A-side,* the other the *K-side.* Similarly the corresponding fore-jars and inlets are designated A and K, respectively (Fig. 1). In cases, when the top concentration is situated at one side of the trough (i.e. when α- and β-gradients are established, cf. Fig. 1) the gradients

are accordingly called A-gradients and K-gradients, abbreviated in the diagrams to GrA or simply A (GrK or K) followed by a figure referring to the ordinal number in an experimental series. The types of gradients demonstrated in Fig. 1, detail 2 b, α_1, and β_1 are K-gradients (GrK). For γ- and η-gradients the designations GrAK or AK are used, when the test solution is supplied to the fore-jar A, and GrEF or EF, when supplied to K. In the first case, AK, equal top concentrations are established along the lateral walls of the test yard. In the latter case, EF, the top concentration is found in the median parts of the yard. The lastmentioned types are demonstrated in Fig. 1, detail 2 b, γ_1 and η_1.

Control conditions, i.e. when pure water only is passed through the apparatus, are designated C with an adhering ordinal number. Accordingly the gradients obtained in the test yard are completely described by simply stating (1) the type of gradient using the symbols, α_1, β_1, γ_1, or η_1. (2) the direction of gradient, e.g. GrA and GrK, and (3) the actual top concentration. This way of putting the matter is applied in the following presentation of the results.

11. *The stability and reproducibility of gradients*

The most appropriate way to define the gradient conditions actually existing in the test yard would be direct measurements *in situ*. It is possible to do this with regard to the determinations of pH. Other agents are analysed in samples taken repeatedly from the downstream ends of the ten longitudinal sections, i.e. just ahead of the test yard. Using a Beckman spectrophotometer, model DU, extinction measurements on the mixtures of sulphite waste liquor (SWL) and tap water are made at 280 mμ. The SWL exhibits a distinct absorption maximum at this wave-length (Aulin-Erdtman, 1949, 1958, p. 149, Fig. 1; Hägglund 1951, p. 211, Fig. 49). The stability and reproducibility of gradients are controlled regularly during the experiments. The results exposed in Table 2 are typical. In this case 20 % SWL tapped at Svanö sulphite pulp mill in November 1958 (cf. Table 7) was used as a model at the rate of flow of 1 cm/sec. Constriction plate 3 (Fig. 5) and gate α_1 (Fig. 1) were employed. Successive samples of the mixtures of liquor and Uppsala water were taken and analysed as described above.

The results compiled in Table 2 (cf. also Fig. 11) lead to the following conclusions. (1) The stability in the course of time is rather high in all cases, with the exception perhaps of GrA 18/12—58. The oscillating values in this case are difficult to explain. (2) The reproducibility is good, irrespective of whether the apparatus is taken to pieces between the measurements. (3) The maximum concentration of the experiment, obtained on December 18th, 1958 under GrK-conditions, is 10 % higher than that obtained under the corresponding GrA. It is a general feature of the present technique that in

spite of the use of the same constriction plate and otherwise identical experimental conditions, neither the top concentration nor the shape of corresponding inversed gradients do really constitute exact mirror images. However, no great differences ever occurred. The deviations must depend on differences in connection with the discharge of water or test solution.

I do not intend to discuss in detail the reasons for these deviations, as they are of little importance for the biological conclusions drawn in the present study.

E. The recording of the momentary positions of fishes

A time lapse filming technique for recording of the momentary positions of the fishes swimming freely about in the test yard was introduced by HÖGLUND (1953). This technique is adopted here. The succession of the instantaneous positions of the test fishes is recorded with the aid of a Paillard, Bolex Rx, film camera. By means of an automatic timer provided with interchangeable gear wheels, exposures are made as a rule every 30 second. When experiments are performed in darkness, the film recording is made possible by means of a Braun automatic electronic flash aggregate, delivering synchronous flashes. These were found to have no considerable influence upon the behaviour of the fishes.

The main advantages of film recording are (1) objectivity and accuracy, e.g. accurate intervals between records; (2) the possibility of preserving primary experimental data; (3) saving of time as the observer can simultaneously concentrate on studying the behaviour of the fishes during the experiment and take notes, (4) the possibilities of studying various types of orientation (see Chapter IV).

III. The performance of experiments

A. The planning and conducting of experimental series

As seen in Figs. 12 and 15 the final experimental routine includes a series of control periods alternating regularly with a series of periods providing gradient conditions, each period usually lasting 30 minutes (cf. also p. 59). Control periods normally succeed two gradient periods with corresponding inversed gradient conditions in close succession. Starting with weaker gradients, successively stronger ones are used.

The test fishes used in a series of experiments, the results of which are assembled into one reaction diagram (cf. p. 56 and Table 1) — always belong to the same species and are approximately of the same size (less than, or about ten centimetres in length). They are taken at random from the populations held in the acclimation aquaria. The number varies from

Table 2. Stability and reproducibility of gradients. The same sulphite waste liquor (XI. 58; cf. Table 7) was used as model at the same standardized conditions. Mean top concentration in italics.

Date for experiment	Kind of α_1-gradation	Sample taken in channel number	Extinction values obtained with the aid of Beckman DU at 280 mμ From December 18th, 1958 and until February 18th, 1959, the apparatus was repeatedly taken to pieces for cleaning. During the two days February 18th and 19th, 1959, it was working all the time															Mean value	Standard error of the mean $\pm \frac{s}{\sqrt{n}}$	Maximal deviation from meam in % of mean value	Number of determinations
18 12 1958	GrA	1	0.406	0.344	0.341	0.342	0.342	0.356	0.362	0.369	0.396	0.397	0.406	0.342	0.331	—	—	*0.364*	0.0077	11.5	13
	GrK	10	0.402	0.402	0.402	0.407	0.407	0.405	0.405	0.405	0.403	0.404	0.401	0.401	0.399	—	—	*0.403*	0.0002	1.0	13
18/2 1959	GrA	1	0.355	0.362	0.354	0.357	0.354	0.350	0.355	0.350	0.352	0.351	0.353	0.349	0.349	0.350	0.346	*0.352*	0.0010	2.8	15
		2	0.354	—	—	0.355	—	—	0.357	—	—	0.348	—	—	0.345	—	—	0.352	0.0002	2.0	5
		3	0.327	—	—	0.330	—	—	0.328	—	—	0.324	—	—	0.318	—	—	0.325	0.0002	2.2	5
		4	0.285	—	—	0.287	—	—	0.290	—	—	0.287	—	—	0.282	—	—	0.286	0.0000	1.4	5
		5	0.237	0.245	0.242	0.244	0.233	0.237	0.238	0.232	0.239	0.244	0.235	0.233	0.236	0.235	0.242	0.238	0.0002	2.9	15
		6	0.186	—	—	0.192	—	—	0.188	—	—	0.192	—	—	0.188	—	—	0.189	0.0000	1.6	5
		7	0.127	—	—	0.133	—	—	0.133	—	—	0.130	—	—	0.125	—	—	0.130	0.0000	3.8	5
		8	0.080	—	—	0.072	—	—	0.078	—	—	0.077	—	—	0.075	—	—	0.076	0.0000	5.3	5
		9	0.022	—	—	0.019	—	—	0.023	—	—	0.023	—	—	0.018	—	—	0.021	0.0000	14.3	5
		10	0.006	—	—	0.003	—	—	0.004	—	—	0.006	—	—	0.002	—	—	0.004	0.0000	50.0	5
19 2 1959	GrA	1	0.353	0.354	0.350	0.350	0.354	—	—	—	—	—	—	—	—	—	—	*0.352*	0.0000	0.6	5
Time for sampling in minutes			0	10	20	30	40	50	60	70	80	90	100	110	120	130	140				

one to ten. Before starting the experiments the fishes are allowed to grow accustomed to the new environment in the test yard for at least two hours or in most cases overnight. During the period of time necessary for the performance of an experimental series they remain overnight in the test yard. Generally the same fishes are used in a whole series. If completed at a later date new specimens are usually tested.

B. **Working procedure**

Before being put into the trough, the glass sheets, the strainer box with glass beads, the gradient generating gate, and the throat constriction gears are thoroughly cleaned. The trough is filled with feed-water and the pressure head corresponding to the drainage desired as well as the water level (11 cm) adjusted. Air is evacuated from the mouth-pieces, from the distribution chamber, and from the throated mixing devices. Marriotte's flask is filled with 9° test solution of a definite concentration. The rubber plug passed by the cooling coils and the air venting glass tube is fixed in the neck of the flask, and the flow cooling water started. The proper constriction plate for regulating the test solution discharges is selected, and the proper screw-lid and adhering outlet tube tightly fastened.

The fish to be tested are put into the test yard. The sheltering hood is placed over the yard, and the desired light condition, light or darkness, is arranged. If an experiment is to be carried out in darkness, the Braun electronic flash aggregate is put into place. The proper gear wheel connected with the automatic releaser of the film camera is applied, i.e. usually that one giving an exposure every 30th second. The time lapse film recording of the movements of the test fishes must not start, before the animals appear to be well adjusted to the experimental cage.

At the beginning of an experimental series the standard conditions chosen, viz. date, species, agent, gate and constriction plate, length of interval between exposures, etc. are distinguished on the recording film by marking exposures. Also the beginning of separate experimental periods are marked out on the film by photographing white sheets of papers on which are painted letters and figures (C 1, A 1, K 1, etc.) corresponding to the indications used in the diagrams (cf. fig. 7).

The steepness of gradient is conveniently changed by replacing the constriction plates in the control box at the outlet of Marriotte's flask. For A- or K-gradients the screw-lid with adhering mouth-piece fitting the distance from the flask to fore-jar A or K, respectively, is used. The stop-cock inserted between the flask and the box is closed, when the screw-lids are exchanged in order to obtain the inversion of an actual gradient. In order to make sure of well established gradient conditions before starting the recording this cock is opened as a routine measure exactly five minutes before the first

exposure, which, during the examination of the film, is considered to introduce the proper gradient period (cf. the vertical arrows in the experimental diagrams of Figs. 11—18). The frame counter dial of the camera is controlled and the starting time noted to the minute before the automatic releaser is started.

During the course of separate experimental periods the characteristic behaviour of the fishes is noted. Necessary pH-measurements are made *in situ,* and necessary samples for chemical analyses are taken.

Chapter II.

The definition of the watery medium in the test yard before and after the addition of the actual test agents

I. General characteristic of feed-waters

The results of the present investigation must necessarily be judged after taking into consideration the qualities of the two kinds of feed-water supplied to the apparatus, viz. Uppsala tap water and Hölle river water.

A. The tap water of Uppsala

The tap water of the town of Uppsala is subsoil water coming from strata of sand, gravel, and pebbles of the esker, called Uppsala-åsen, which stretches through the town. The quality of this water is shown in Table 3. The analyses were carried out by the technical staffs at the Institute of Public Health in Stockholm and at the Water Laboratory of the Waterworks of Uppsala, in accordance with instructions published by the Royal Medical Board of Sweden (Medd. fr. Kungl. Med. Styr. Nr. 87, 1950).

The data in the first column of the table are based on ten samples collected at different points within the water-supply net of the town, viz. the pumping stations at Islandsfallet and Stadsträdgården, as well as from peripheral taps. The samples are taken on different occasions during the years 1942, and 1951—1957. When only a single figure is given, all analyses have given identical results. Otherwise the values fall within the range determined by the extreme figures given for every specific analysis. As the majority of the experiments reported on in the present investigation were performed during the years 1958 and 1959 the corresponding analyses on two occasions, viz. December 16th 1958 and December 3rd 1959, during this time are given in the next two columns of Table 3.

On account of the considerable variation in the conductivity given in the table a series of electrolytic conductivity determinations at 10° C was performed on the tap water daily during the period March 2—10, 1960. A 4886-S conductivity bridge manufactured by the Leeds & Northrup Company, Philadelphia, provided with a conductivity cell, type Phillips GM

4221, was used. The mean value from nine determinations and the corresponding standard error of the mean is 551.3 ± 0.9 (range 548—557) $\varkappa \cdot 10^6$.[1]

The tap water of Uppsala is practically free from bacteria. It is supplied unchlorinated and altogether untreated. The total hardness is notable and is due to the occurrence of Palaeozoic limestone formations within the area covered by the Uppsala esker. According to Winkler analyses the content of dissolved oxygen is near zero, as is typical for subsoil waters. In later years, i.e. since the end of 1956, artificial infiltration of water from the river Fyris into the esker has been carried out. With the possible exception of the content of nitrites no effects of this procedure could be observed in the water analyses during the last years or from 1958 onwards. Considering the low oxygen content of the water the nitrate values are remarkably high. However high nitrate content, viz. 5—8 mg/l, is found as a rule in all analyses from 1942 with one exception for the year 1957. Analysis according to MULLIN and RILEY (1955) of the amount of nitrate nitrogen was also performed at the Institute of Limnology, Uppsala.[1] 6.90 mg NO_3 per litre was found. This is in agreement with the data given in Table 3. The temperature of the water in the laboratory was somewhat higher than the figures given in the table, or varying between 8—9° C independent of season.

The extraordinarily constant quality of the tap water of Uppsala with regard to all examined constituents is most suitable for the present investigation. This is valid for the whole period 1942—1959 as well as for the course of any single year.

B. **The feed-water used at Hölle Salmon Research Laboratory**

In the experiments with Uppsala water mixed with HCl or sulphite waste liquor, a gradation with regard to pH as well as to the concentration of free CO_2 is brought about by the addition of hydroxonium ions to the bicarbonate content of the feed water. In order to get a quantitative estimate of the rôle played by the two kinds of agents, pH and P_{CO_2}, prevailing in these types of experiments some supplementary tests had to be carried out, with the aid of a water containing essentially less bicarbonate.

For this reason the whole apparatus was moved to the Salmon Research Laboratory at Hölle situated on the river Indalsälven in the province of Jämtland. The water in question (see Table 3) is surface water accumulated in the big dam built in connection with Hölleforsens hydro-electric power-station near the laboratory and the nearby breeding plant for salmon young. The intake is some metres below the surface.

The experiments at Hölle were carried out between July 15th and August 15th 1959. Fortunately enough this summer was extraordinarily dry and hot.

[1] For the performance of these measurements my thanks are due to Mr Thorsten Ahl, of the Institute of Limnology at this University.

Table 3. Main constituents of the two kinds of feed-waters. Data compiled from 13 different reports on water quality analyses. Where not given, quantities are expressed in mg/l.

	Uppsala water			Hölle water
	10 occasions 1942 1951—57	Dec. 16, 1958	Dec. 3, 1959	Aug. 3, 1959
Physical examinations				
Temperature, °C	7—8	7	7.5	18
Colour, mg/l Pt	< 5	< 5	< 5	15
Turbidity	0	0	0	light particles
Odour, strength at 40°C	0	0	0	slight
» , kind	0	0	0	aromatic
Residue after sedimentation during 24 hours	0	0	0	considerable
Chemical examinations				
$KMnO_4$ consumed	< 5	3	3	19
Residue on evaporation	324—396	—	—	—
» after ignition	250—340	—	—	25
Electrolytic conductivity, $\times x10^6$	342—555	367	577	—
pH	7.1—7.6	7.4	7.6	7.5
NH_4	0.1	0.1	0.1	0.1
Ca	95—117	103	119	9
Total hardness, German degrees	13.3—16.4	14.4	16.7	1.3
Fe	< 0.1	< 0.1	< 0.1	0.12
Mn	< 0.06	< 0.05	< 0.05	< 0.05
HCO_3	**259—313**	**307**	**315**	**20**
Cl	17—31	29	31	2
SO_4	24—38	39	40	no reaction
NO_3	< 2—8	9	5	< 2
NO_2	< 0.02	0.05	0.04	< 0.01
PO_4	< 0.1	< 0.1	< 0.1	< 0.1
Bacteriological examinations				
Number of colonies per ml on gelatin plates after 48 hours at 20°C	0	0	—	—
Fermentation test in lactose broth at 37°C. The most probable number of *coli-aerogenes*-bacteria per 100 ml	< 1	—	—	—

During the performance of the experiments there was no rain to cause high turbidities in the river water. Thus the feed-water passing through the apparatus remained particularly clear, though it contained some suspended organic matter. The experiments could be kept running without interruption for at least two days and nights before the accumulation of mud in the strainers conspicuously influenced the flow through the apparatus. Then the equipment had to be cleaned.

During the period of the experiments the temperature of the Hölle water rose day by day from about 16° C late in July to about 20° C in the middle of August. During the period of observation the drop of temperature during the night never exceeded 1° C.

A water sample was taken on August 4th, and examined at the Institute

of Public Health in Stockholm. The results are given in Table 3. Oxygen determinations according to Winkler performed on July 20th indicated full saturation at 18° C. Oversaturation was avoided by the heavy injection of air into the water supplied to the laboratory.

II. The calculation of P_{CO_2}

A. The problem

Knowing the concentration of any component of the carbonic acid protolytic system, viz. either the concentration of carbonate ions, that of bicarbonate ions, or the amount of carbon dioxide, the other two constituents and the total CO_2 are defined, provided that the actual pH is known.

The following equilibria exist:

$$\begin{matrix} CO_2 + H_2O \\ \uparrow\downarrow \rightleftharpoons HCO_3^- + H^+ \rightleftharpoons CO_3^{2-} + 2H^+ \\ H_2CO_3 \end{matrix} \qquad (1)$$

After adding increasing amounts of a strong acid to a natural water containing originally a definite concentration of bicarbonate, the solution will gradually contain increasing amounts of molecularly dissolved CO_2, which in time will escape. This is the result of the appearance of increasing amounts of hydroxonium ions (decreasing pH-values). In order to obtain in the experimental cage of the fluviarium a graded environment with regard to the CO_2-concentration as well as to pH, pure HCl is added to the actual kind of feed-water used in an experiment, i.e. either to Uppsala tap water containing a fairly high bicarbonate concentration (310 mg/l) or to Hölle water with minute amounts of bicarbonate (20 mg/l). See further p. 37! The hydrochloric acid is introduced into the proper mixing box, and diluted there by the stream of feed-water to a definite concentration. The hydroxonium ions react with the bicarbonate ions in the water. The protolysis of bicarbonate forming carbonic acid presumably occurs instantaneously in the mixing box. The main part of the carbonic acid is further dehydrated to free CO_2 and water, and a new state of equilibrium within the system (1) is attained within a few minutes (Harvey, 1957, p. 155; Hägg, 1959, p. 113). The diluted acid containing dissolved CO_2 is then apportioned into the ten longitudinal sections of the open through, and spread over the test yard in accordance with the ordinary distribution principle applied in the present technique (cf. p. 24). In this process it will meet more uncontaminated water and the consequence mentioned will be repeated. Quantitatively these rather complicated reactions cannot be followed in detail. Attention will be concentrated on the environmental conditions actually prevailing in the test yard, or rather at the entrance of the latter. With a knowledge of pH and the definite bicarbonate content of the original feed-water the CO_2-concentration in each

section can be calculated provided equilibrium is attained after the different mixing procedures and provided no CO_2 is lost from the liquid phase during the passage of the acidified water through the apparatus all the way to and past the test yard.

The transference of carbon dioxide from the acidified feed-water flowing through the fluviarium was expected to take place rather slowly (HARVEY, 1957, p. 155, and p. 182). In order to get a rough idea about this question some crude preliminary determinations were made.

A vessel with plain vertical walls and an open area of about 6 dm^2 was filled to the same hight, viz. 11 cm, as that prevailing in the test yard. Uppsala tap water, acidified with hydrochloric acid to pH about 3, was used. It was left standing in a cooling bath in order to keep the temperature of the stagnant fluid constant at the proper experimental temperature of 9°—10°C. Samples were taken on successive occasions, and the total remainder of carbon dioxide was determined. The total carbon dioxide concentration of the acidified tap water was found to decrease relatively slowly. Thus after 5 minutes the decrease was about 6 %, after half an hour about 25 %, after one hour about 33 %, and after two days about 60 % of the initial values. The loss found here is certainly higher than in the test yard with continuously supplied acidified tap water, since in the latter case the atmosphere close above the water surface certainly contains larger amounts of CO_2 than normal.

If this is true the neglect of the carbon dioxide escaping from the liquid phase into the air, "the gas exsorption" according to GUYER & TOBLER (1934a), during the time of the passage of the fluid through the experimental flume will not introduce an unduly large error into the calculation of the quantities of dissolved CO_2 obtained. The approximation seems reasonable on account of the fact that the flow is viscous, and consequently only a thin surface layer comes into contact with the atmosphere during the time necessary for the flow through the open parts of the apparatus.

The assumption is furthermore justified by the low exsorption rate of CO_2 established by GUYER and TOBLER (1934 a, b) in a thorough investigation of this special problem. According to these authors the dominant factor determining the rate of gas exsorption is the gas transport within the liquid phase, which is in turn defined by the aid of Fick's law for diffusion (JACOBS, 1935, p. 9 ff.).

The same kind of reasoning — though conversely — will be valid for the absorption of oxygen from the air into the Uppsala water, poor in oxygen, flowing through the apparatus. This is a condition precedent for the experiments with concentration gradients of dissolved oxygen which are performed in the present study (cf. Chapter V).

As shown in Table 4, no considerable variation of pH occurred at different points along the open through. This fact indicates that (1) the pertinent

Table 4. pH measured *in situ* in five sections at equal distances along the open trough. Supply conditions: 1-C HCl; constriction plate with diameter of aperture 1.2 mm; gate α_1; GrA; Uppsala water.

Section number	Time for determination, Aug. 8, 1960	Upstream distance in cm from the rear net of the test yard					
		86	61	48	33	18	3
1 (A) ...	14h18′	5.73	5.76	5.75	5.76	5.76	5.78
	14h41′	5.59	5.60	5.60	5.59	5.61	5.60
3 (C) ...	14h23′	5.95	5.96	5.95	5.96	5.95	5.84
	14h46′	5.82	5.82	5.75	5.76	5.88	5.88
5 (E) ...	14h28′	6.27	6.28	6.26	6.26	6.23	6.19
	14h52′	6.14	6.12	6.11	6.12	6.15	6.10
7 (G) ...	14h32′	6.65	6.66	6.65	6.65	6.66	6.62
	14h57′	6.59	6.58	6.59	6.59	6.63	6.62
9 (I) ...	14h37′	7.25	7.25	7.25	7.28	7.26	7.25
	15h02′	7.22	7.22	7.22	7.23	7.24	7.25

chemical reactions have attained their equilibria before the acidified water mixtures enter the open channels in the middle part of the trough, and (2) no considerable amounts of dissolved carbon dioxide escape from the solutions during their passage through this part of the flume.

Accordingly, though new equilibria of the carbonic acid protolytic system appear in the ten sections of the trough, the total amount of CO_2 within the liquid phase of each section is approximately unchanged. Or in other words, the ten watery mixtures occurring in the test yard all contain different fractions of carbon dioxide as bicarbonate and carbonate ions, as unhydrated molecules of CO_2, and as carbonic acid, all in equilibrium with each other and with the hydrogen (hydroxonium) ions present, as expressed by equations (1).

The latter can be determined by pH-measurements *in situ*. The problem can now be summarized as follows: (1) An adequate estimate of the total amount of carbon dioxide in Uppsala and Hölle water must firstly be gained. For that purpose the concentration of one constituent at least of the carbonic acid system ought to be determined. The bicarbonate concentration which is the predominating part in both feed-waters, will receive special consideration. (2) The actual concentration of free CO_2(H_2CO_3 included) ought to be calculated for different pH-values. The relationship between pH and the concentration of CO_2 transformed into P_{CO_2}-values expressed in mm Hg ought to be stated with regard to Uppsala and Hölle water. (3) Finally, the accuracy of the results obtained by this procedure of calculation will be verified by alkalinity determinations on samples taken from the longitudinal sections just ahead of the test yard with prevailing gradient conditions.

B. The pH-measurements performed

The pH-meter used is a directly indicating model, Radiometer PHM 24, Copenhagen, operated by built-in batteries (cf. Fig. 2). A glass electrode, type G 202 A, is used in combination with a calomel electrode, type K 401, for reference. When measuring pH-values higher than 8, a special glass electrode, type G 202 B, is used. The pH is measured by dipping the electrodes into the water flowing through each longitudinal section, just ahead of the front net separating the test yard from the subdivided middle part of the experimental flume.

In some cases thé mere arithmetical mean value of different pH-values is used. This procedure is not mathematically correct, but implies inconsiderable errors as long as the differences in pH are small (Turell, 1946; Österlind, 1949, p. 99).

C. The bicarbonate content of the feed-waters

The relative high content of bicarbonate in the tap water of Uppsala is utilized for obtaining combined pH- and steep CO_2-gradients (cf. Chapter V). The mean value from alkalinity determinations [1] with high accuracy according to Berger (see Karlgren, 1958) on five samples of this water taken from the middle of the test yard and performed in April 1959 was 309.5 at pH 7.4. This value agrees well with the bicarbonate concentrations arrived at during later years in the analyses performed at the municipal Waterworks of Uppsala (Table 3). Samples taken in December 1958 and December 1959 gave the values 307 mg/l at pH 7.4 and 315 mg/l at pH 7.6, respectively. Consequently the bicarbonate concentration of the feed-water used in Uppsala can be estimated to *310 mg/l* or $5.1 \cdot 10^{-3}$ *mol/l* at *pH 7.5*. As can be seen from Table 3 the bicarbonate concentration of the supply water used in the experiments carried out at the Hölle Salmon Research Laboratory is about *20 mg/l* or $3.3 \cdot 10^{-4}$ *mol/l* at *pH 7.5*.

These figures will be used in the calculations of (1) the total CO_2 (cf. Table 5 on p. 38) and (2) the relationship between pH and carbon dioxide tension of P_{CO_2} with regard to the two kinds of feedwater used in the present study.

D. The relationship between pH and P_{CO_2}

The fundamental equations for the quantitative relationship between the constituents of the carbonic acid protolytic system are found everywhere in the reports (e.g. Buch, 1945, 1951; Österlind, 1949; Harvey, 1957; Davenport, 1958). A conventional system of symbols will be used in the following discussion:

[1] These determinations were performed at the Institute of Limnology at this University. The author is obliged to Professor W. Rodhe, the head of the institute.

c (with an index) means the concentration of a substance in mol/l.

K'_1, pK'_1 „ the "apparent" or "incomplete" dissociation constant of carbonic acid and its negative logarithm, respectively.

c_s „ the carbon dioxide concentration expressed in mol in the liquid phase in equilibrium with the CO_2 tension of 1 atmosphere in the gas space above.

The law of mass-action regarding the protolysis of HCO_3^- is changed into the logarithmic form as expressed by Henderson—Hasselbalch's equation:

$$pH = pK'_1 + \log \frac{c_{HCO_3^-}}{c_{CO_2}} \qquad (2)$$

In this expression c_{CO_2} includes the sum of the concentrations of hydrated carbon dioxide or H_2CO_3 and molecularly dissolved CO_2. The former constitutes about a thousandth of the latter (HÄGG, 1959, p. 50).

According to Henry's law the free carbon dioxide obtained in the solution may be expressed in P_{CO_2}. This is the partial pressure in mm Hg that CO_2, if in equilibrium with the actual amount of dissolved CO_2, would exert in the gas phase above the solution.

On this account the denominator of the last term in equation (2) can be replaced by $c_s \cdot P_{CO_2}$. Further, if the carbonate content is disregarded as being very small at pH lower than 7.5 in relation to that of the bicarbonate, the latter can be expressed according to (3).

$$c_{HCO_3^-} = c_{totalCO_2} - c_{CO_2} \qquad (3)$$

Substituting the right-hand term in equation (2) we get

$$pH = pK'_1 + \log \frac{c_{totalCO_2} - c_s \cdot P_{CO_2}}{c_s \cdot P_{CO_2}} \qquad (4)$$

Table 5. Total carbon dioxide and numerical values of the constants used in the calculation of the relationship between pH and P_{CO_2} for Uppsala and Hölle water as shown in fig. 6.

Uppsala water	K_1' (10°C) $=3.39\times10^{-7}$; $pK_1'=6.47$; (BUCH, 1945, p. 15, 1951, p. 6)
	c_s (10°C) $=0.054$; (BUCH, 1945, p. 14. Data from BOHR, 1897, according to FOX, 1909, p. 10)
	$c_{total}=5.48\times10^{-3}$ mol/l.
Hölle water	K_1' (20°C) $=3.98\times10^{-7}$;[1] $pK_1'=6.40$; (BUCH, 1951, p. 6)
	c_s (20°C) $=0.039$; (cf. above)
	$c_{total}=3.53\times10^{-4}$ mol/l.

[1] In BUCH (1945, table 2 on p. 15) 3.27×10^{-7} is wrong, presumably a printer's error (cf. BUCH, 1951, p. 6).

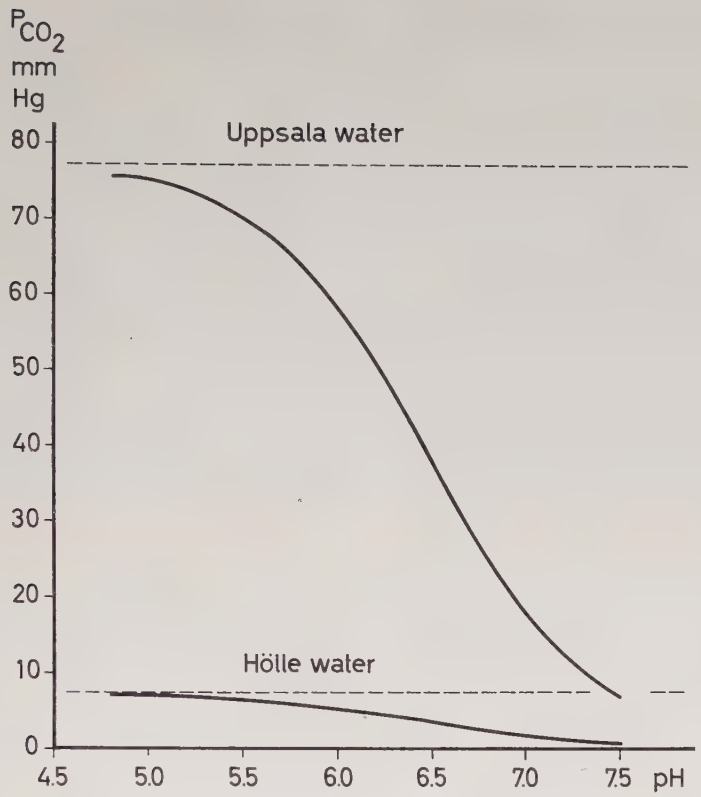


Fig. 6. The relationships between pH and P_{CO_2} calculated for the two kinds of feed-water according to expression (4) on p. 38. The maximum P_{CO_2} values which can be obtained by the acidification of Uppsala and Hölle water are demonstrated by horizontal lines of short dashes.

In this final equation only pH and P_{CO_2} are unknown. The numerical values of the constants are given by Buch (1945, 1951) (cf. Table 5). As pH is measured *in situ* the corresponding P_{CO_2}-values in atmospheres can be calculated according to (4). These values are multiplied by 760 to get P_{CO_2} expressed in mm Hg.

The relationships between pH and P_{CO_2} for the two actual kinds of feed-water are presented graphically in Fig. 6. All P_{CO_2}-values presented in diagrams published in this work are based on pH-values measured *in situ* and taken from the calibration curves presented in this figure.

E. The accuracy of the calculation

The validity of the P_{CO_2}-values arrived at by the procedure of calculation described above is controlled by accurate titrometric analyses according

to BERGER (cf. KARLGREN, 1958) of the samples taken from the fluviarium under well defined graded pH-conditions.[1]

The results are compiled in Table 6. The following experimental conditions occurred. 1.0 C HCl was introduced into fore-jar A from Marriotte's flask provided with the constriction plate having a diameter of aperture of 1.2 mm. At the same time 9° Uppsala tap water was supplied to the apparatus with gate α_1 in place. This was made at the usual drainage of 20.5 l./min. corresponding to a linear flow rate of c. 1 cm./sec. From each longitudinal section just ahead of the front net confining the test yard, samples were siphoned into Winkler flasks which were tightly plugged and left overnight. After the different degrees of acidification of the feed-water obtained in this way the remaining bicarbonate ions of 3 to ten ml of each sample were titrated with 0.02032 C HCl. The pH-values simultaneously measured *in situ* are corrected to 20°C. The calculation of the constituents of the carbonic acid system is made with the highest accuracy. The

Table 6. Quantities of carbon dioxide constituents in different parts of the experimental trough during graded pH-conditions in Uppsala water. The data are based on titration alkalinity determinations performed on samples taken *in situ*.

Sample taken from	Ml titrator solution consumed pro ml sample 1)	pH 9° 2)	pH 20° 2)	Titration alkalinity (BERGER) meq/l	HCO_3 mg/l	P_{CO_2} 3)	CO_2 saturation 4)
Section 1 (A).....	0.003	5.4	5.3	0.061	4	— (71)	—
2 (B).....	0.003	5.5	5.4	0.061	4	69 (70)	276
3 (C).....	0.023	5.7	5.6	0.467	29	65 (66)	260
4 (D).....	0.050	5.9	5.8	1.016	63	60 (61)	240
5 (E).....	0.085	6.2	6.1	1.727	105	48 (50)	192
6 (F).....	0.130	6.4	6.3	2.642	161	40 (42)	160
7 (G).....	0.168	6.6	6.5	3.514	214	32 (33)	128
8 (H).....	0.205	6.9	6.8	4.166	253	20 (21)	80
9 (I)	0.241	7.2	7.1	4.897	298	11 (12)	44
10 (K).....	0.248	7.3	7.2	5.039	307	9 (10)	37
The middle of the yard prior to the addition of acid...	0.250	7.35	7.25	5.080	309.4	8.4(9)	33

1) Mean value from 2—3 analogous determinations performed by different persons. The volume of the sample titrated was 10 ml for sections 1—3, 6 ml for 4—5 and 3 ml in the remaining cases

2) Mean value from determinations made on five different occasions.

3) The figures within brackets refer to values obtained from calculations based upon the bicarbonate concentration of the original feed water

4) Saturation in relation to the mean tension of the atmosphere, viz. 0.25 mm Hg

[1] These analyses were performed at the Institute of Limnology, University of Uppsala.

ionic strength is taken into consideration and advantage taken of the relevant constants given by Buch (1945) for different temperatures and salinities.

The titration values arrived at for the samples from sections 1—2 seem to be too high. This is presumably in consequence of a titration error on the relatively small sample. As will be seen from the data given in the table the calculations based upon the bicarbonate concentration of the original feed-water give P_{CO_2}-values which differ very little from those arrived at on the basis of the alkalinity determinations performed on samples taken *in situ*. This demonstrates the accuracy of the calculation method generally used. Furthermore the P_{CO_2}-values arrived at may be compared with those determined by McClendon (1917) for pure bicarbonate solutions at different alkalinities and pH-values as given graphically by Pia (1933, Fig. 4, p. 58). The P_{CO_2}-values taken from this graph (partly by extrapolation for lower pH-values) satisfactorily agree with those presented here.

It appears also from this kind of analysis that no considerable amount of CO_2 escapes from the liquid during the passage from the mixing box to the test yard.

As may be expected the values calculated in Fig. 6 are somewhat too high, but not more than five to ten per cent even with regard to the lower P_{CO_2}-values.

III. Definition of original sulphite waste liquor (SWL)

The SWL:s used in the present study all come from Svanö sulphite paper pulp mill, Svanöbruk. This pulp mill applying the calcium bisulphite pulping process produces exclusively sulphite pulp from spruce wood (*Picea excelsa*) of three main qualities which may be characterized by their relevant Roe numbers.[1] The wood raw material is as a rule totally decorticated (1-ma in Table 7), occasionally decaying wood of poorer quality (dec. w. in Table 7). On the whole, apart from some exceptions, SWL:s of equal qualities were obtained. Renewed supplies were sent in tightly closed 60 l barrels of polythen plastic. They were filled directly from the digesters or from storing basins in the mill containing concentrated liquor. Before use in the present experiments the liquors were stored in the closed barrels

[1] Lignin takes up large quantities of chlorine and other halogens. The Roe number or chlorine number is defined as the amount of Cl_2 in grams that will be absorbed, when pulp drenched in water is treated with the gas. The Roe number is thus a value for the chlorine consumption of the pulp, i.e. an approximate measure of its lignin content (Häggland, 1951, p. 420). High Roe numbers indicate that the delignification is not completed as the wood is not cooked so long time.

Table 7. Chemical and technological characteristics of the original SWL:s from Svanö Sulphite paper pulp mill which were used as test substances.

Time for tapping	I. 57	II. 57	III. 57	VII. 57	VIII. 57	X. 57	VI. 58	IX. 58	XI. 58	I. 59	III. 59	VII. 59	Reference for method employed
Time for analysis	July 57			Nov. 59	Jan. 58		Sept. 58	Nov. 59					
pH *	2.9	3.2	3.1	1.7	4.6	4.6	2.5	2.7	2.8	2.8	2.3	3.0	
$\varkappa$. $10^{-2}ohm^{-1}cm^{-1}$ *	0.94	0.94	0.80	1.43	0.7	0.75	0.88	1.01	0.91	1.12	1.07	0.92	
Total solids, g 1000 g Volatile substances not included.* ..	116	122	110	105	66.2	58.8	117	122	119	185	119	133	C. C. A. 22 (1948)
Residue on ignition g/1000 g *	16.6	17.3	10.1	12.0	12.3	12.5	14.2	16.0	15.7	26.4	16.7	17.6	[1]
Specific gravity g cm^3 *	—	—	—	1.04	—	—	1.04	1.05	1.05	1.08	1.05	1.06	
Free SO_2, iodometrically, g/1000 g *	—	—	—	0.7	—	—	0.42	1.2	0.9	1.8	1.7	0.7	Sieber (1943, p. 262)
SO_2, totally, iodometrically, g/1000 g *	6.2	6.2	3.5	4.2	1.2	1.2	4.3	6.3	5.3	10.4	7.2	5.7	Sieber (1943, p. 263)
S, totally, g 1000 g*.....	8.2	9.5	5.2	7.7	4.8	4.5	6.7	7.7	8.1	8.6	9.3	7.3	Sieber (1943, p. 267)
Ca, g 1000 g *	5.8	6.0	5.0	4.3	4.0	4.2	4.9	5.5	5.5	9.0	5.9	6.1	[2]
Mg, mg/1000 g *	16	52	57	56	50	38	92	86	78	128	75	89	Sandell (1950, p. 419) [2]
Fe, mg/1000 g *	65	31	20	26	29	23	23	21	31	46	29	23	Sandell (1950, p. 375) [2]
Methoxyl content, g/l ** .. .	—	—	—		—	—	—	—	—	—	—	9.5	
Lignin content, g l **	—	—	—		—	—	—	—	—	—	—	60.0	Calculated from the methoxyl content (Hägglund, 1951, p. 224)
Reducing sugar, g/l **	—			—		—		—	—	—	—	34.8	Acc. to Schorl, C.C.A. 11
$KMnO_4$-consumption, g/l ** ..	—	—	—		—	—		—	—	—	—	350	
BOD_5(20°C), g l **		—	—			—	—		—	—	—	48.9	Am. Publ. Health Ass. (1955, p. 260)
Extinction at 280 mμ of 1000 ppm orig. SWL diluted in Uppsala tap water	—	—	—	0.64	0.53	—	0.84	0.78	0.80	0.99	0.69	0.74	Beckman spectrophotometer, Model DU.
Roe number ***	8.5	8.5	8.5	8.5	9.0	9.0		7.0	5–6	5–6	8.5	9.9	Cf. Hägglund (1951, p. 214 and p. 420)
Wood quality ***	1-ma	1-ma	1-ma	1-ma	dec. w.	1-ma	—	1-ma	1-ma	—	1-ma	1-ma	

* Analyses performed at the Central Analytical Laboratory, Institute of Chemistry, University of Uppsala.
** „ „ „ „ Skogsindustriernas Vattenlaboratorium, Stockholm.
*** Information from Svanö paper pulp mill.
[1] Residual inorganic matter collected after burning the total residue on exaporation 1 hour at 550°C.
[2] Ca, Mg, Fe are determined in the ash after separation of SiO_2; Ca precipitated as oxalate; Mg and Fe colourimetrically by titan yellow and o-phenanthroline, respectively.

at room temperature for periods ranging from a few days to several months. As far as posssible one single batch of SWL was used in every series of experiments.

The liquors were not standardized before use as test solutions. Each supply of raw SWL was subjected to chemical analysis. The analyses were performed mainly at the Central Analytical Laboratory at the Institute of Chemistry at this University and at Skogsindustriernas Vattenlaboratorium, Stockholm. In this way the SWL:s used as test solutions must be considered as rather well defined within the limits of what is practically possible.

The data obtained from the chemical analyses are presented in Table 7. It can be stated that the analysed constituents and other characteristics of the present SWL:s fall well within the ranges of the corresponding data given in the extensive literature on this field (e.g. Schwalbe and Becker, 1919; Klason, 1920; Heiduschka and Munds, 1929; Hägglund, 1928, 1951; Vogel, 1939, 1948; Tyler *et alii*, 1946; Adler, 1947; Warrick, 1947; Stockman and Hägglund, 1948; Reiff and Haas, 1949; Rudolfs, 1953; Meinck, Stoof and Kohlschütter, 1956; Rennerfelt, 1958; Nylander and Rennerfelt, 1960).

In the following descriptions of SWL gradient conditions in the test yard the concentrations are given in ppm of original SWL as defined in Table 7. Since different liquors are used in various experimental series they will be referred to in every special case by the time for tapping using the designations contained in Table 7.

In connection with the reaction diagrams presented in Chapter V also the pH-values and extinction-values at 280 mμ (Beckman DU spectrophotometer) of the highest concentration of different gradients are given.

Chapter III

Presentation and quantitative estimation of the experimental results

I. Introduction

Different arrangements as to the length of experimental periods, the mutual succession of periods, and the frequency of exposures (observations), have been tried in the early experiments in order to develop an appropriate experimental routine (cf. Figs. 11—18). The results of the differently arranged experiments, mainly concerning SWL, are nevertheless given in full in the present publication. Later on working with combined pH/P_{CO_2}-gradients of α_1-type a certain scheme is as a rule followed in all experimental series (cf. Fig. 12). In the experiments with dissolved oxygen longer test periods than the usual 30 minutes are required (Fig. 17).

The characteristic behaviour displayed by particular test animals with the present technique are discussed in greater detail in Chapter IV. In the present chapter stress will be laid upon the quantitative estimation of the preference reactions shown in different kinds of gradients as compared with those in unadulterated water ("control condition").

The extensive observation material collected must be published in a very concise form and yet be as perspicuous and comprehensive as possible. For this purpose a few standard types of graphical presentations are used, covering the characteristic features inherent in the primary observations. More than 100,000 film frames have been examined. This corresponds to about 840 hours' effective experimental time. Up to ten fishes were used at the same time (cf. Table 1). Accordingly, the results shown in the diagrams including all observational data in an abbreviated form, are based on a half to one million recorded momentary positions of fishes in the test yard.

There is a considerable variation in the intensities of the reactions obtained in the same graded environment. This is demonstrated in the graphs by the dispersion of dots representing so called reaction values (p. 48). This is further elucidated by a more penetrating analysis of some experiments especially regarding the reactions of *Leuciscus rutilus*.

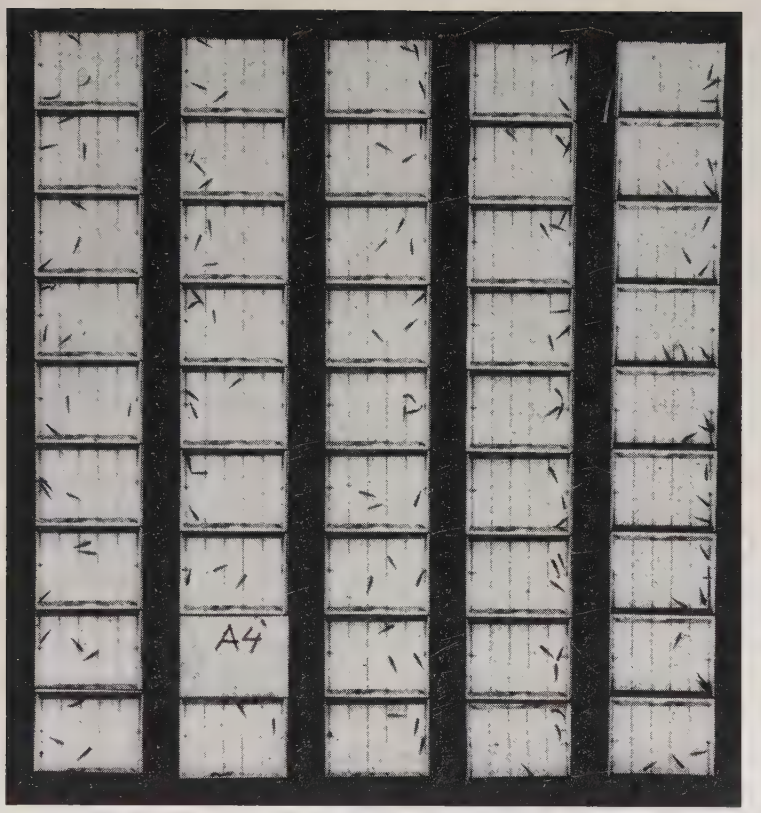


Fig. 7. A sequence of frames from a part of the recording film of the experimental series considered in Fig. 41 in which steeper and steeper α_1-gradients of SWL (IX. 58; cf. Table 7) were established in Uppsala water. The momentary positions of the same three intact *Phoxinus phoxinus* are recorded at 30-second intervals. The last eight minutes of one gradient period (K4) and the first 14 minutes of the next following period (A4; cf. the marking exposure) with the corresponding, inverse gradient conditions are shown. The top concentration was about 1500 ppm. The marking exposure covers a period of five minutes during which the new gradient was established.

The test yard is projected on each frame with the A-side to the left. The parallel lines painted on the bottom show the borders between every other concentration step. Starting from the upper left-hand corner, the course of the experiments is followed by examining the frames downwards in the column on the extreme left, and so on. It is clearly shown that the fishes prefer to stay in the less SWL-contaminated parts of the test yard.

The whole test area is well covered, when projected on a frame of the 16 mm substandard-film (Fig. 7). The examination of the film is conveniently carried out with the aid of a Kodagraph film reader, model MPF, provided with a special attachment for the reading of substandard-film. The projection of the frames produces an enlargement of about twenty

times the original. Thus the records of the fishes are easily distinguishable. If the projection of a fish extends over more than one section, it is assigned to the section which includes the tip of the snout.

II. The primary treatment of experimental data

A. Frequency histograms of observed visits in different sections of the test yard

The first step in the quantitative analysis of the *preference reactions* shown by the fishes is similar to that used by HÖGLUND (1953) and LINDAHL and MARCSTRÖM (1958). But contrary to the procedure described in the latter paper time lapse filming and essentially longer test periods are used. The film is studied frame by frame and the number of observed visits in every section of the test yard, successively numbered 1—10 (cf. Fig. 1), are added up. The sum obtained from every consecutive sequence of ten frames (usually exposed at 30 second intervals) is calculated, i.e. corresponding to each five minute period. The same procedure is repeated for the whole experimental period, lasting usually 30 minutes. The rather inconsiderable parallax due to refraction and the error on account of undue projection of the fishes against the delineations on the bottom of the test yard are disregarded.

The distributions of records over the width of the test yard obtained at various standard conditions in the yard are taken from the films and presented as *frequency histograms*. The heights of the piles represent the number of observed visits in each section calculated as percentage of the total number of observations (n) as stated by a figure on the bottom left of every histogram.

B. The meaning of the mean position value(s) (mpv)

A *mean position value*, expressed in section number as unit, is calculated for the actual periods of an experimental series in the following way. The number of observed visits within each section is multiplied by the corresponding section number. The sum of the ten products obtained is then divided by the total number of observations. The mean position values calculated in this way are usually written in the abbreviated form *mpv* with an index giving the length of time on which the calculation is based. Mpv related to periods lasting for 5 and 30 minutes are thus designated mpv_{5min} and mpv_{30min} respectively.

The actual mpv is indicated in the frequency histograms by a dotted vertical line and/or by a figure on the bottom right. The standard error of mpv is based upon the pertinent mpv_{5min}.

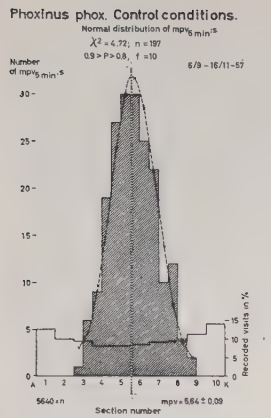

Phoxinus phox. Control conditions.
Normal distribution of mpv5 min:s
χ² = 4.72; n = 197
0.9 > P > 0.8, f = 10
6/9 – 16/11–57
Number of mpv5 min:s
Recorded visits in %
5640 • n
mpv = 5.64 ± 0.09
Section number

Fig. 8.

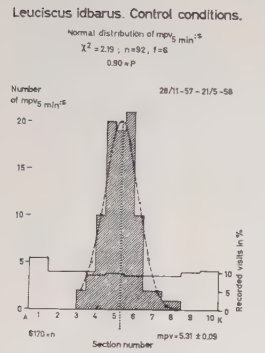

Leuciscus idbarus. Control conditions.
Normal distribution of mpv5 min:s
χ² = 2.19; n = 92, f = 6
0.90 • P
28/11–57 – 21/5–58
Number of mpv5 min:s
Recorded visits in %
6170 • n
mpv = 5.31 ± 0.09
Section number

Fig. 9.

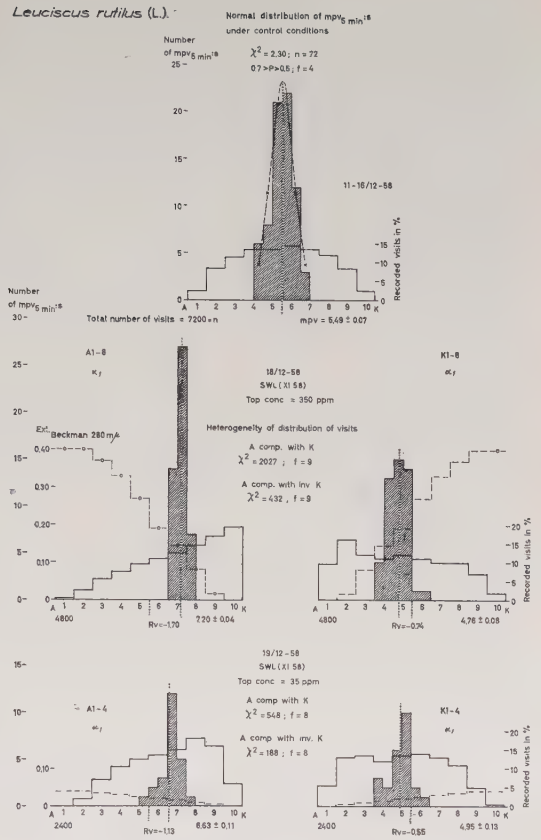


Fig. 10.

Fig. 8. Preferred parts of the test yard shown by three *Phoxinus phoxinus* under control conditions not preceded by gradient ones. The blank histogram shows the distribution of visits in the hypothetical ten sections of the test yard. The striated area shows the distribution of mpv_{5min}. This is compared with the next coinciding normal distribution (curve of short dashes) and the pertinent statistics are given (cf. p. 65). The mpv for the total observational time (16 hours, 25 minutes) is represented by a vertical line of short dashes.

Fig. 9. Preferred parts of the test yard shown by seven intact *Leuciscus idbarus*. Records were taken in pure Uppsala water for a total of 7 hours and 40 minutes at about 2 cm/sec. In all other respects the ordinary control conditions prevailed.

Fig. 10. Histograms as in Fig. 8. Ten intact *Leuciscus rutilus* recorded in pure Uppsala water for a total of 6 hours (uppermost histograms). The same specimens were also tested in corresponding, inversed α_1-gradients of SWL (XI. 58; cf. Table 7) of two different strengths, viz. for 4 hours each in GrA and GrK (dashed stepped curves connected to the middle histograms) with the top concentration of about 350 ppm ($Ext_{Beckman}$ at 280 mμ of about 40 units), and for 2 hours each in GrA and GrK with the top concentration of about 35 ppm (lowermost histograms). For the explanation of the statistical data see p. 65 f. Cf. also Figs. 11, 42, and 45.

C. The symmetrical dispersion of mpv_{5min} under control conditions

A number of experiments was run with the same experimental conditions for periods longer than 30 minutes. The dispersion of mpv_{5min} are in some of these cases inserted in the frequency histograms of observed visits, and marked by striated or wholly black areas (Figs. 8—10, 25, and 31—34).

Considering the mpv_{5min} from a large number of control periods not preceded by gradient periods, the material is grouped together for each twentieth of the width of the test yard and arranged as histograms as seen in Figs. 8—10. This procedure is applied to *Phoxinus phoxinus, Leuciscus idbarus,* and *L. rutilus,* all being species which characteristically swim about more or less continuously in the test yard. The first aim is to find out how closely the frequency distribution of mpv_{5min} fits a normal distribution. Using the method described by BONNIER and TEDIN (1957, p. 52) the nearest corresponding normal distribution is calculated and plotted in the figures. The coincidence between the actual frequency distribution and the corresponding normal one is then estimated with the aid of χ^2-analysis (BONNIER and TEDIN, 1957, p. 70). The results of the analyses are entered in the relevant figures.

Judged from the data in Figs. 8—10 the divergence from the normal distribution can be attributed to chance only. For the three species studied in this respect the means of the normally distributed mpv_{5min} coincide very closely with the median line of the test yard. This leads to the conclusion that mpv obtained during control periods not preceded by gradient periods tend to almost coincide with the median line, if suitably long periods are run. This in turn indicates that possible small assymmetries in the experimental arrangements of control periods do not considerably influence the permeation of all parts of the test yard.

D. The meaning of the reaction value(s) (rv)

The appearances of mpv_{5min} during control periods intercalated between gradient periods (cf. e.g. Figs. 12 and 13) show that the fishes cannot be entirely unaffected by the preceding experimental elements. Thus it is not really a question of true control values. On this account the preference reactions during a gradient period are not compared with the mpv obtained during the immediately preceding control period. The *strength of reaction* is defined as the *lateral displacement of the average distribution (mpv) from the median line* of the test yard. This is furthermore justified by the above finding, viz. that mpv during real control conditions tend to coincide with the median value of the sum of the section numbers, i.e. 5.5. The deviation of the mpv obtained during certain gradient conditions from

this value is defined as the *reaction value (rv)*. The maximum attainable rv is accordingly 4.5. This means that all records are found one of the two lateral sections (number 1 or 10). The significance of different behaviour patterns for the evaluation of rv is discussed more closely in Chapter IV (Fig. 24). An index figure connected with the symbol, rv, indicates the length of the relevant test period. In order to counteract the effects of possible training and/or accidental technical inequalities and disturbances, the reaction value is generally based upon two corresponding inversed gradient periods in close succession, and of the same duration. This procedure is especially important, when testing more stationary species (cf. e.g. Fig. 51).

The kind of preference reaction in relation to the direction of the gradient is described by the use of the following terms, viz. *avoidance, attraction,* and *indifference*. This is done without respect to underlying physiological mechanisms and possible toxic effects. *Avoidance* (with a negative sign) simply means that the recorded visits in the higher concentrations are less frequent than in lower ones. The contrary is *attraction* (with a positive sign). When the graded conditions are found to exert no significant influence upon the frequency distribution in comparison with control conditions, *no reaction* or *indifference* is said to occur.

III. Graphical presentation of the results

A. Diagrams showing the course of experiments and experimental series

The momentary positions of the fishes as recorded (usually at 30 second intervals) on the film frames reflect the preference reactions during different experimental periods (cf. Fig. 7) as do in a more concentrated form the successive mpv_{5min} obtained from consecutive lots of ten frames. For a visual survey of the events during the course of an experiment or series of experiments, mpv_{5min} is plotted against experimental time in the form of *experimental diagrams*. After an examination of the film-recorded positions of the fish it was found that five minute periods were most suitable for this purpose.

The construction of the experimental diagrams may be exemplified by Fig. 12. Here, the preference reactions of seven intact roaches are demonstrated in a series of experiments with steeper and steeper α_1-gradients of pH/P_{CO_2}. The width of the trough is represented by the distance between the two horizontal limiting lines. The A-side (cf. Fig. 1) is found at the bottom, and the K-side at the top of the figure. The median line of the test yard is represented by a median horizontal line in the graph. Different experimental periods providing C-, GrA-, and GrK-conditions are separated by vertical lines. The figures on the top concentration side within each

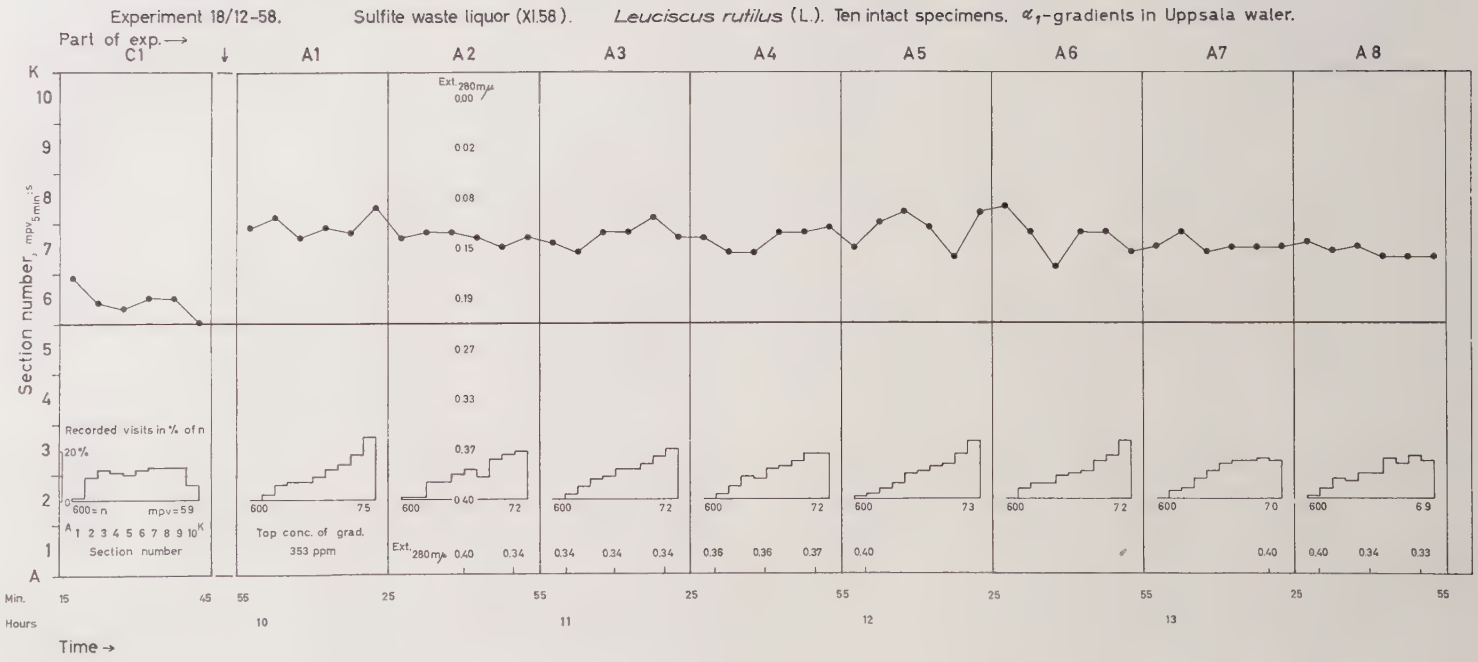


Fig. 11 a.

Fig. 11. Experimental diagram (cf. p. 49) showing the preference recations of ten intact roaches during the course of a series of successive experimental periods lasting for 30 minutes each. C1 and C2 mean control periods with pure Uppsala water; A1—8 and K1—8 periods with corresponding inverse α_1-gradients of SWL (XI. 58; cf. Table 7) with the top concentration of about 350 ppm. The primary observations on the recording film of this experiment are studied from various view-points (cf. Figs. 10, 19, 21—23, 42, and 45).

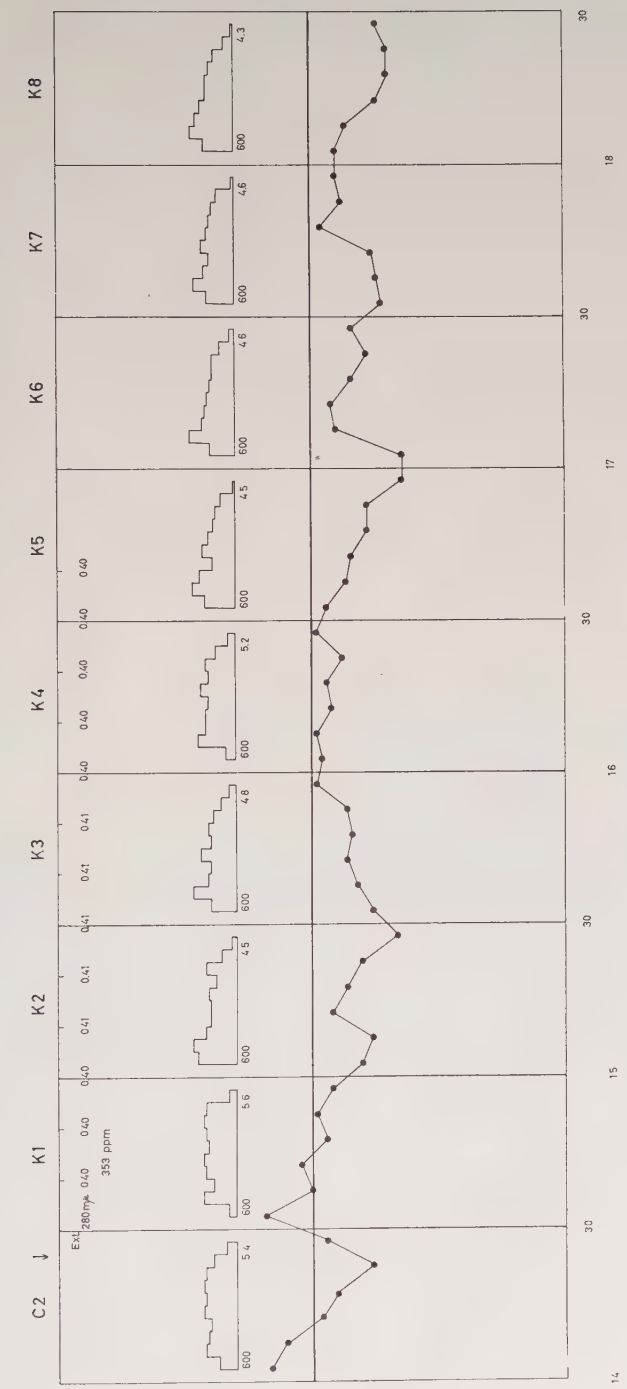

C2
K1
K2
K3
K4
K5
K6
K7
K8
Ex1
280 mμ
353 ppm
600
5.4
5.6
4.5
4.8
5.2
4.5
4.6
4.6
4.3
0.40
0.41
14
15
16
17
18
30

Fig. 11 b.

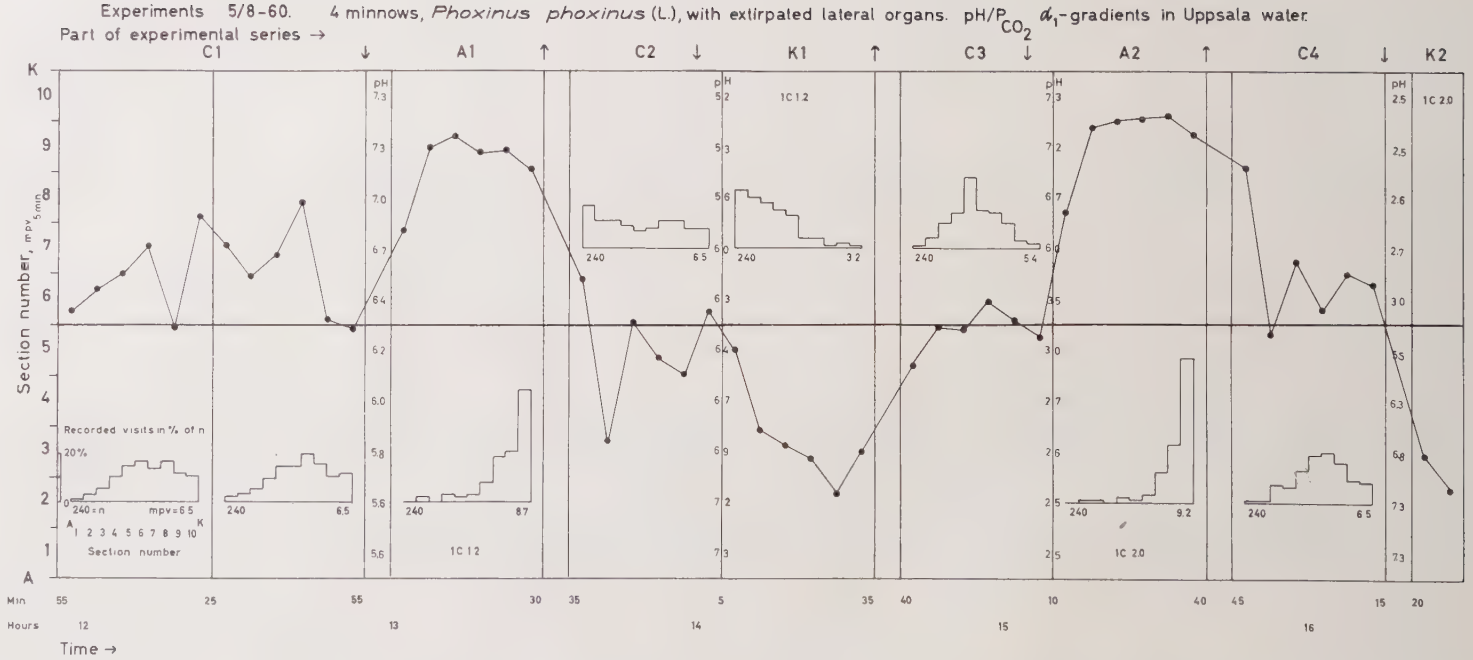


Fig. 15 a. Minnow.

Fig. 15. Experimental diagram (cf. p. 49) showing the preference reactions of specimens with extirpated lateral organs in α_1-gradients (A1—A2 and K1—K2) of pH/P_{CO_2} and in pure Uppsala water (C1—C4). (*a*) Minnow, (*b*) roach, (*c*) salmon parr.

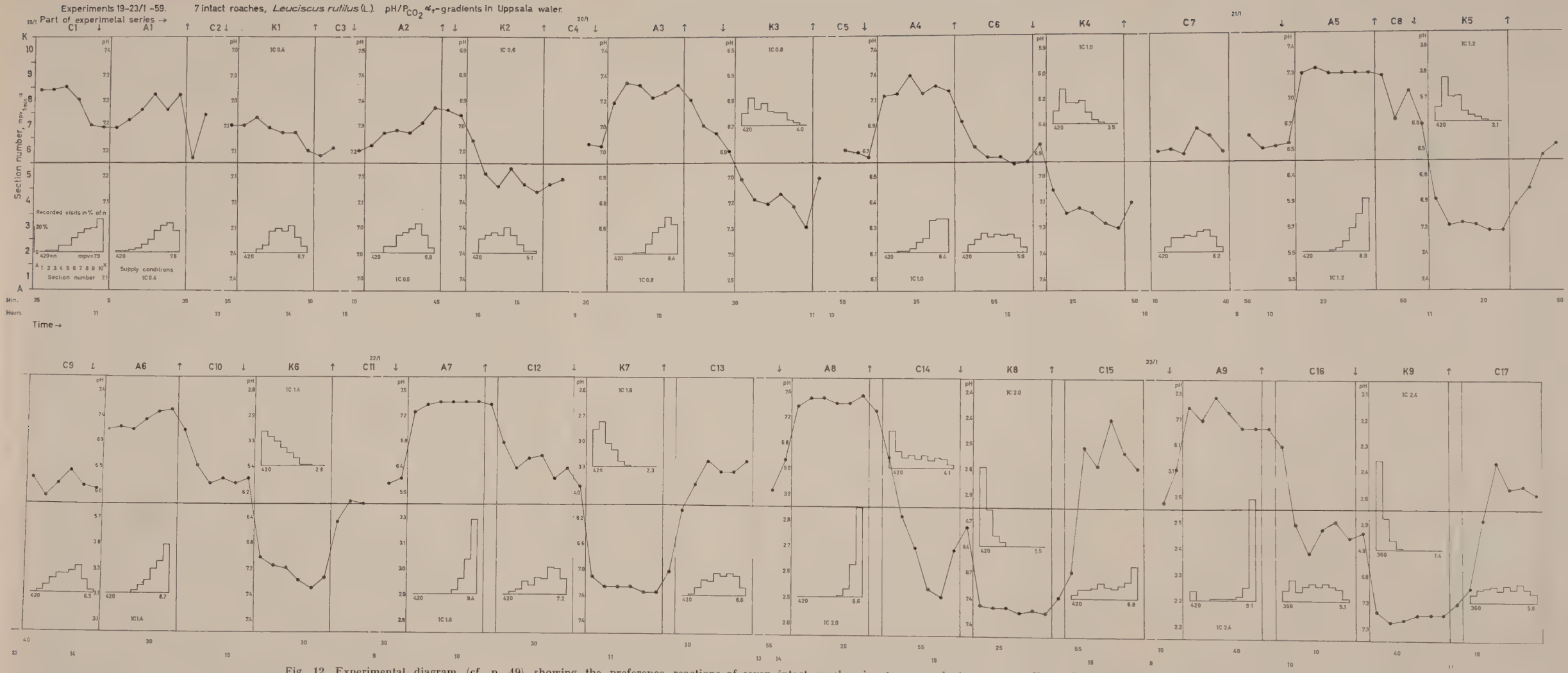

Experiments 19–23/1 –59. 7 intact roaches, Leuciscus rutilus (L.). pH/P_{CO_2} α_1-gradients in Uppsala water.
Part of experimetal series →
Section number, mpv 5 min⁻¹
Recorded visits in % of n
Supply conditions
Section number
Min.
Hours
Time →

Fig. 12. Experimental diagram (cf. p. 49) showing the preference reactions of seven intact roaches in steeper and steeper α_1-gradients (A1—A9 and K1—K9) of combined pH/P_{CO_2} in Uppsala water and during intervening control periods (C1—C17). Using the pH values measured *in situ* the corresponding P_{CO_2} values can be taken from Fig. 6. Cf. also Figs. 29, 31, and 36.

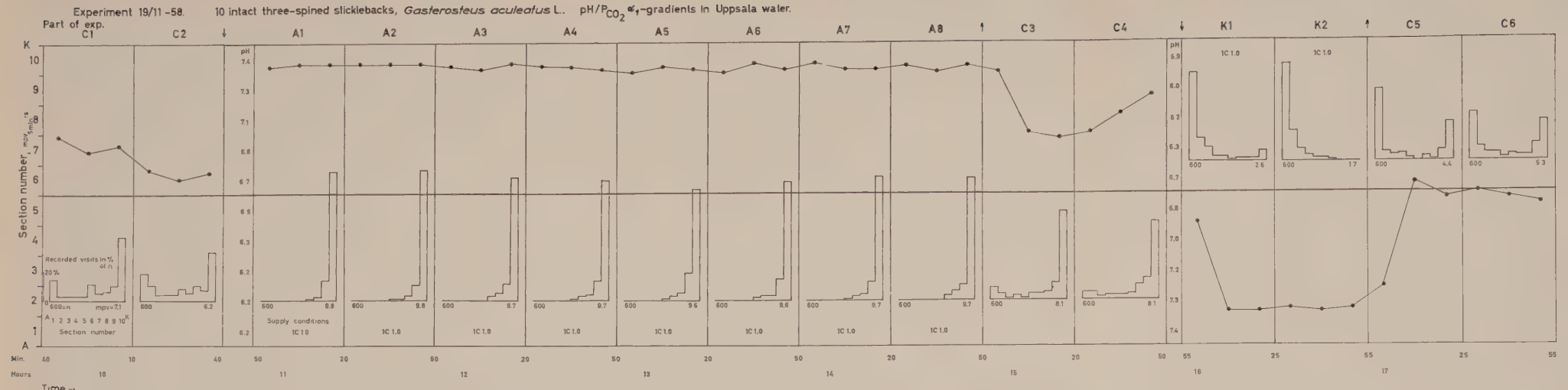

Experiment 19/11-58. 10 intact three-spined sticklebacks, Gasterosteus aculeatus L.. pH/P_{CO_2} α_1-gradients in Uppsala water.
Part of exp.
C1 C2 A1 A2 A3 A4 A5 A6 A7 A8 C3 C4 K1 K2 C5 C6
Section number, mpv5min
Recorded visits in % of n
Section number
Supply conditions
1C 1.0
Min.
Hours
Time

Fig. 13. Experimental diagram (cf. p. 49) obtained with ten three-spined sticklebacks tested in α_1-gradients (A1—8 and K1—2) of pH/P_{CO_2} and in pure Uppsala water (C1—C6). Mpv_{10min} are calculated.

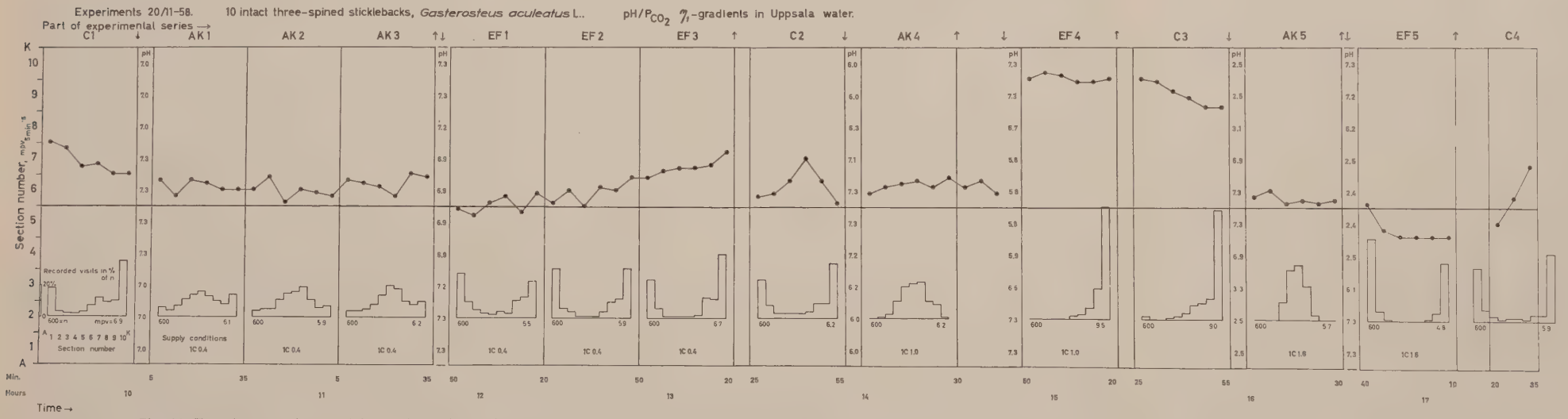

Experiments 20/11-58. 10 intact three-spined sticklebacks, Gasterosteus aculeatus L.. pH/P_{CO_2} γ_1-gradients in Uppsala water.
Part of experimental series
C1 AK1 AK2 AK3 EF1 EF2 EF3 C2 AK4 EF4 C3 AK5 EF5 C4
Section number, mpv5min
Recorded visits in % of n
Section number
Supply conditions
Min.
Hours
Time

Fig. 14. Experimental diagram (cf. p. 49) obtained with the same fish as in Fig. 13, this time tested in γ_1-gradients (AK1—AK5 and EF1—EF5) of pH/P_{CO_2} and in pure Uppsala water (C1—C4).

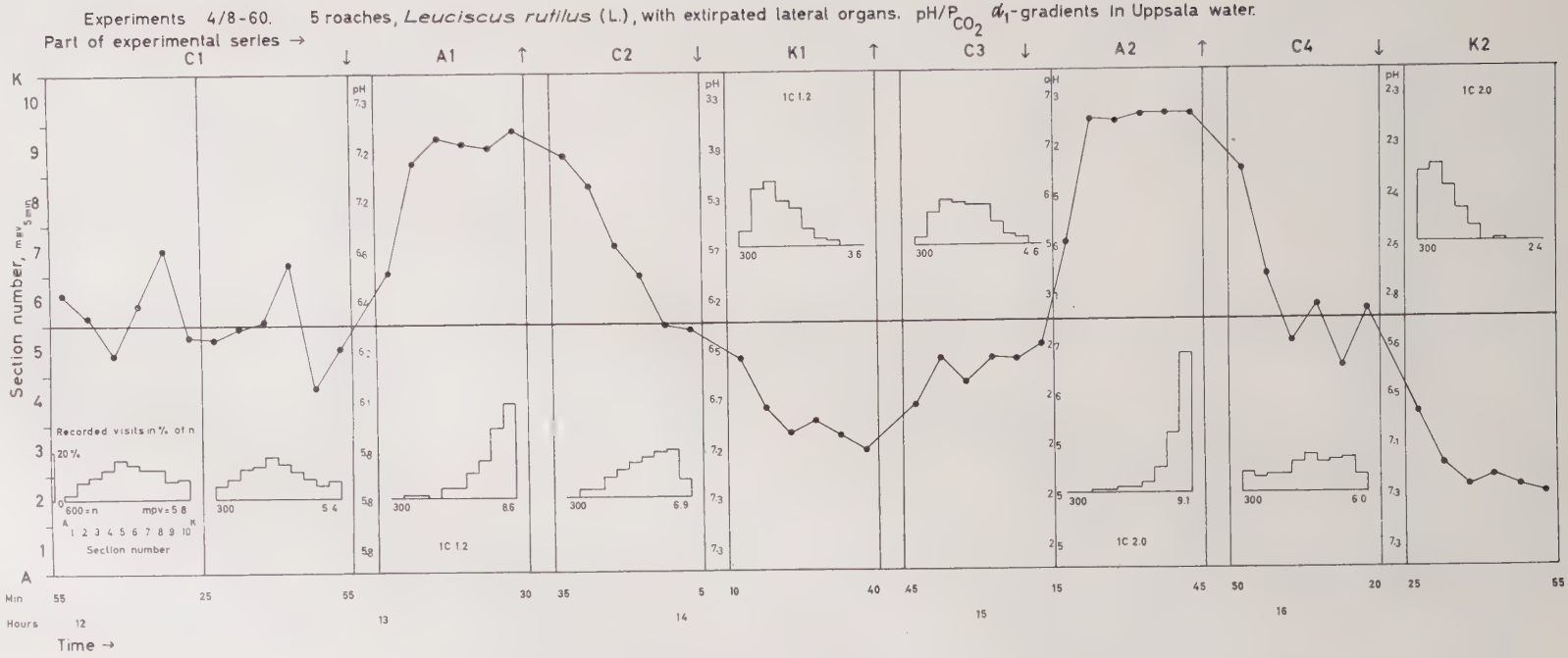


Fig. 15 b. Roach.

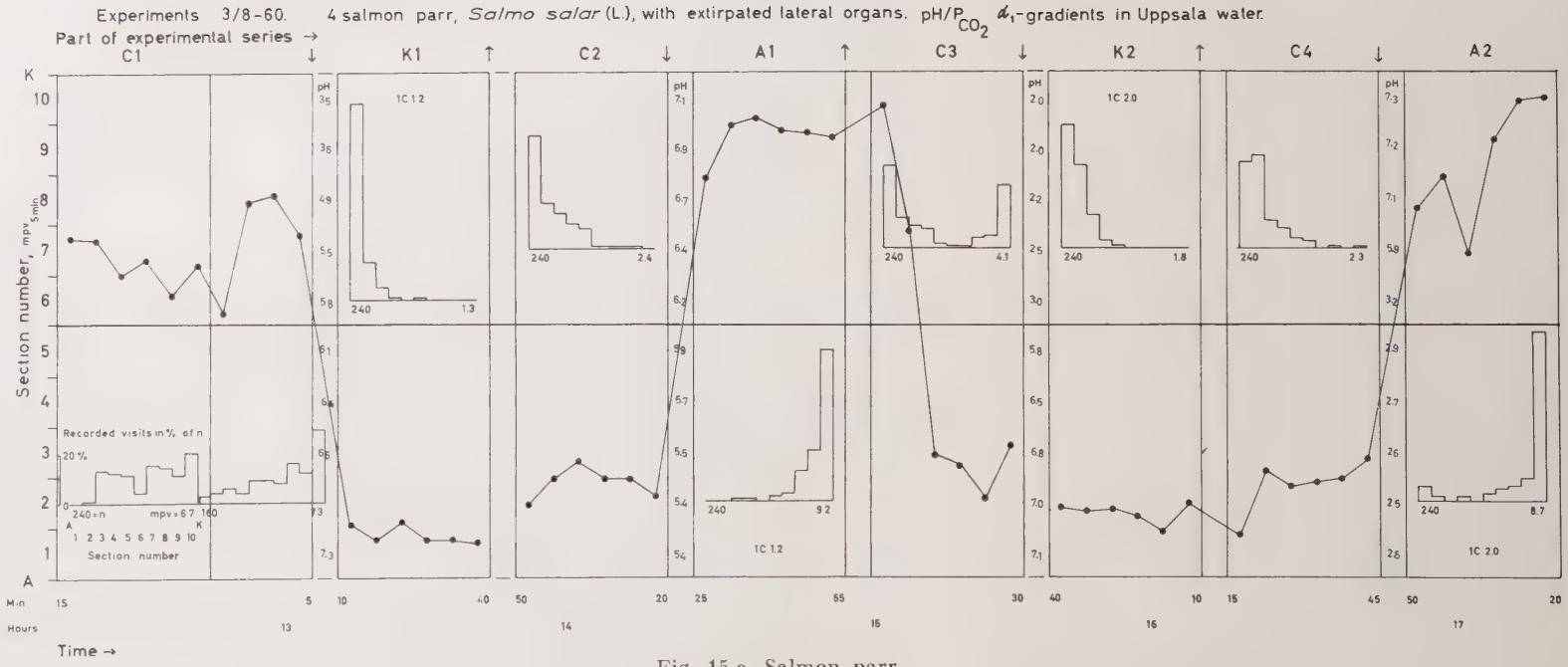


Fig. 15 c. Salmon parr.

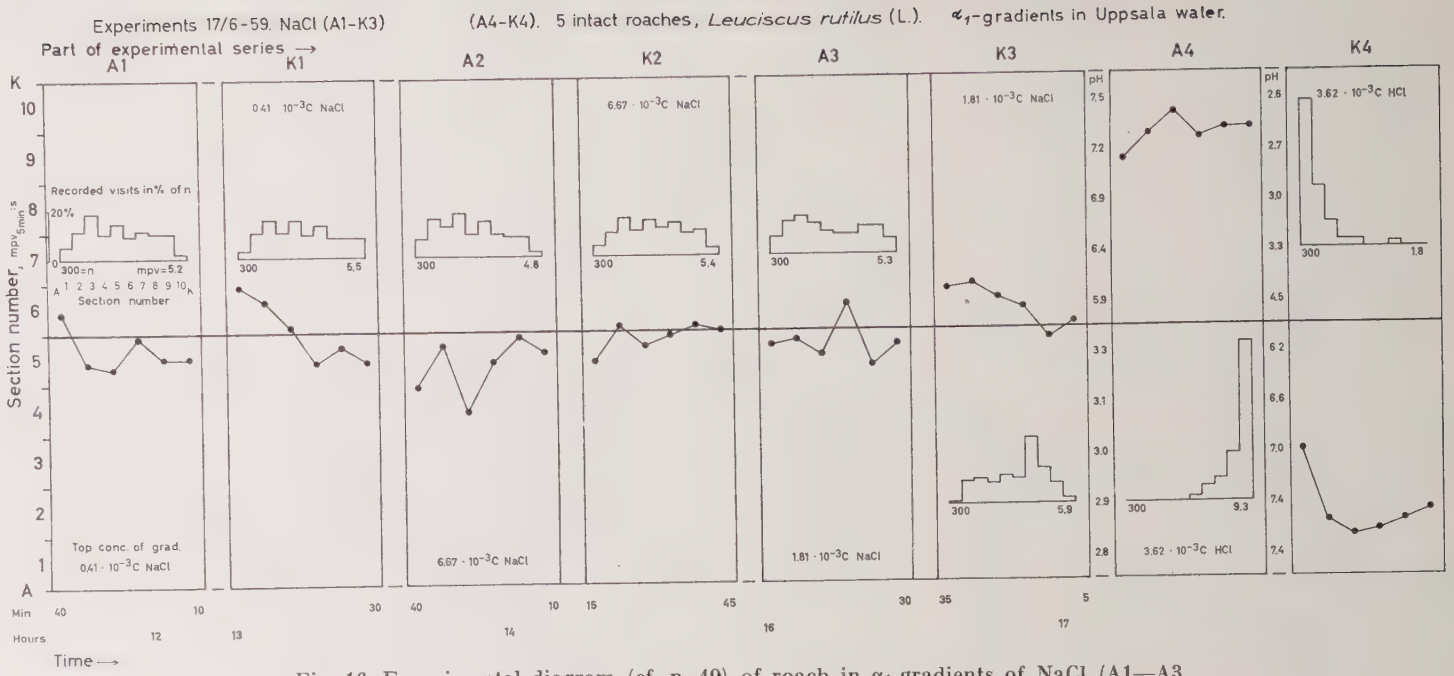


Fig. 16. Experimental diagram (cf. p. 49) of roach in α_1-gradients of NaCl (A1—A3 and K1—K3) and pH/P_{CO_2} (A4 and K4) established in Uppsala water.

frame corresponding to a gradient period, viz. 1C 0.4, 1C 0.6, etc., furnish the particulars with regard to the test solution supplied from Marriotte's flask. For instance, 1C means 1-C HCl, and 0.4 the aperture diameter (in mm) of the constriction plate (Figs. 1 and 5). The arrows at the upper contour indicate the moments of beginning and terminating the supply.

Every dot in the actual diagram (Fig. 12) representing a mpv_{5min} is based upon 70 observations. The six mpv_{5min} within a 30 minute period show the variation (constancy) of the reaction during the course of time with the same standardized experimental condition. Adjacent pairs of dots are joined by straight lines which together constitute a continuous polygonal curve which is continuous as long as the fishes are continually, time-lapse photographed. The recording is started well before the establishment of the actual gradient conditions and continued until their complete extinction. This occurs within less than five minutes after the cessation of the test solution supply. At the interruptions of the continuous filming the curves and outlines are marked with short dashes.

The intensity and stability of the reactions in the course of time are clearly shown, and questions like the following ones can be answered by a glance at the experimental diagrams. How soon after the introduction of a gradient do the preference reactions commence? In which way do the reactions cease at the end of an experimental period?

However, the polygonal curve does not demonstrate the distribution of the records. For that purpose frequency histograms, in which the A-side is always to be found on the left, are inserted in connection with different experimental periods. It must be observed that the coordinates of the polygonal curves and those of the histograms are not identical.

The necessity of considering also the distribution of the observed visits in different sections is clearly demonstrated in the experimental diagram in Fig. 14. Ten three-spined sticklebacks are tested in combined pH/P_{CO_2} gradations in Uppsala water, when gate η_1 is used. In this case, though the frequency distributions are exactly mutually reversed, the mpv are situated near the median line, for example during EFl-3 as well as during AK5.

Lack of space prevents, however, the publication of all experimental diagrams. Some instructive examples are given here representing different species and test agents (Figs. 11—18). A still more concise presentation is therefore consistently used.

B. **Reaction diagrams and reaction curves**

In the elaboration of the results all primary data deriving from the films is first entered into experimental diagrams. This observation material is then rearranged in the more concentrated form of *reaction diagrams*. These (Figs. 26, 29—30, 36—37, 39—44, and 47—51) summarize all quantitative data obtained in the gradient experiments performed.

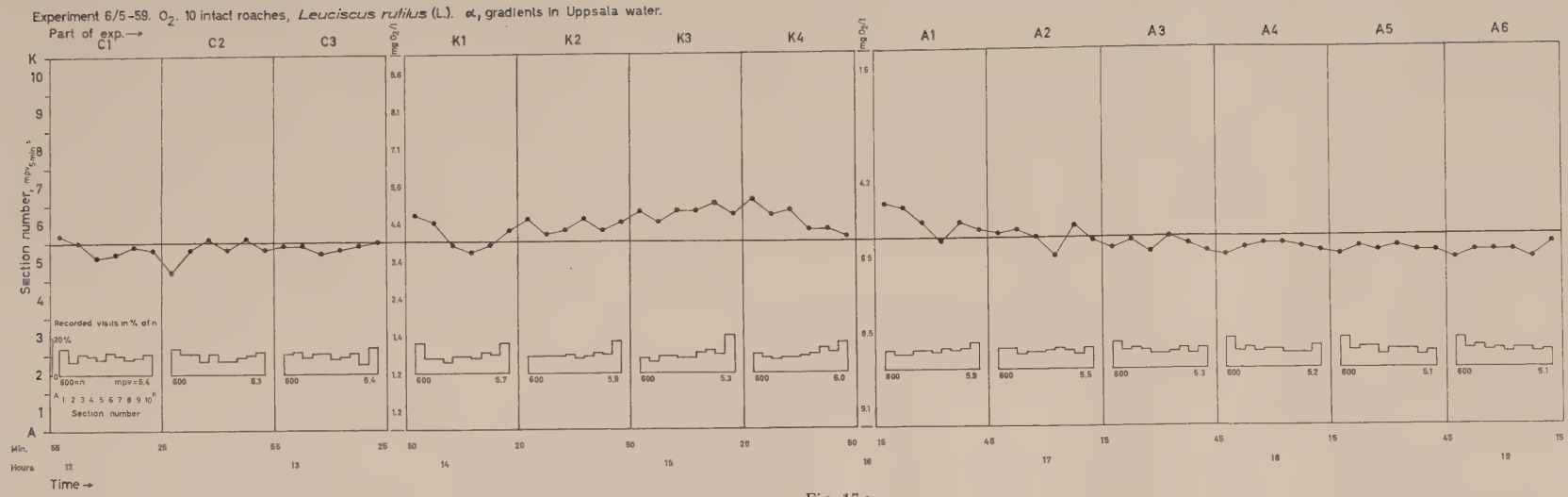


Fig. 17 a.

Fig. 17. Experimental diagram (cf. p. 49) of roach in (*a*) α_1-gradients and (*b*) β_1-gradients of oxygen.

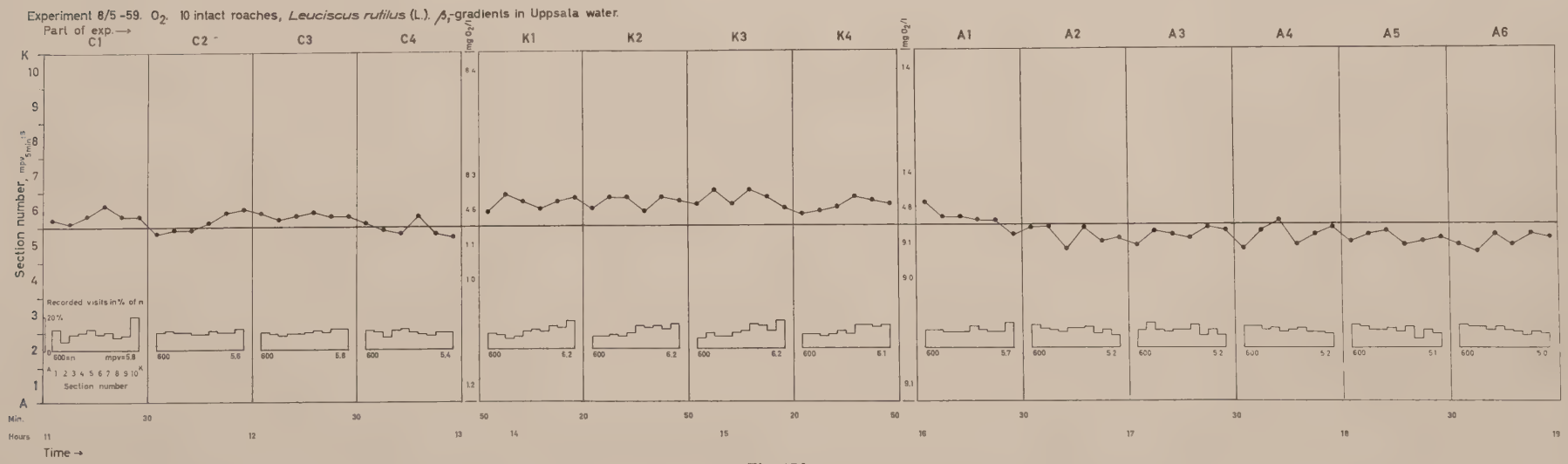


Fig. 17 b.

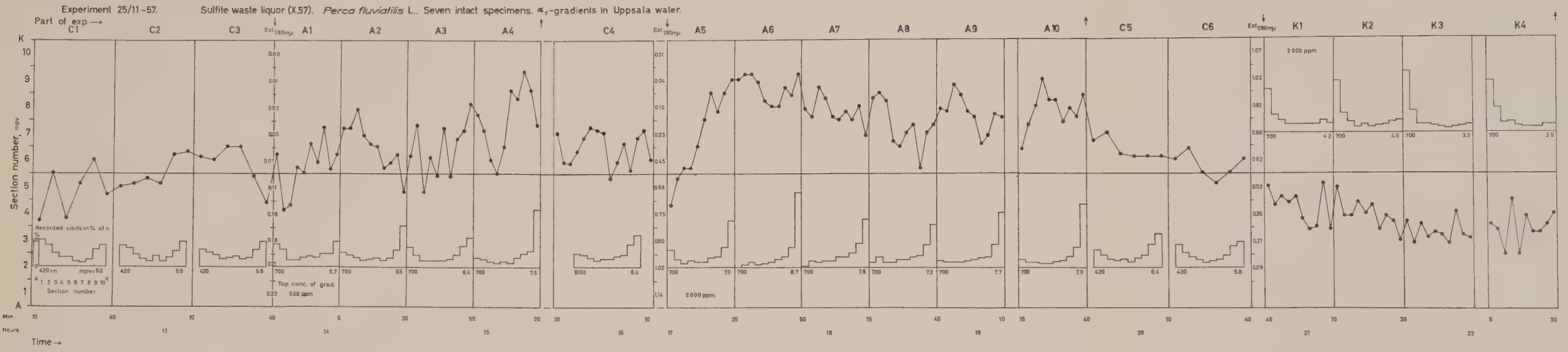


Fig. 18. Experimental diagrams (cf. p. 49) of perch fry in α_1-gradients of SWL (A1—4, A5—10, and K1—4) and in pure Uppsala water (C1—C6). Mpv_{150sec} are calculated.

All the rv_{5min} (expressed in section breadths) obtained with a definite standard gradient are plotted against the actual top concentration, or in the cases when two corresponding inversed gradients were used, the mean top concentration. The latter is expressed in different units, viz. in log ppm (SWL), pH, mm Hg or degree of contamination as defined on p. 99 (CO_2), and mg/l (O_2).

Dealing with the controls, mpv_{5min} is generally plotted against section number which is shown at the extreme left of most reaction diagrams.

The steeper and steeper gradients are in Figs. 36, 37, 39—44, and 47—51 thought of as projected in the graphs perpendicularly to the median line of the test yard, with the steepest ones to the right. Independent of direction (GrA or GrK) the gradients are thought to be situated with their top concentrations constantly at the bottom horizontal line which thus corresponds to the lateral wall on the top concentration side of the test yard. Accordingly concentration steps decreasing toward zero at the upper line (opposite wall of the yard) are found along every "gradient line". This presentation brings about positive rv (attraction) below and negative rv (avoidance) above the median horizontal line. Rv_{5min} are represented by dots. In addition the means of all rv_{5min} from the particular gradients are represented by larger signs (usually circles).

The relationship between the intensity of reaction and the steepness of gradient is illustrated in these graphs by a heavy line, called a *reaction curve*. This is in most cases drawn free hand on the visual representation derived from the plotted rv.

A comparison of the reaction diagrams makes it clear that the shapes of the reaction curves are more or less characteristic of the species and agent tested. They give an idea about the actual *preference threshold value* which might be looked for at the intersection of the actual reaction curve with the median line of the graph.

C. Serial diagrams of frequency histograms

The results are in some cases presented also in the form of *serial diagrams of frequency histograms*. This type of graph shows the distributions at different standard conditions of the visits recorded in the hypothetical sections of the test yard (Figs. 25, 28, 31—34, and 45—46). It is used as a complement to certain reaction diagrams.

IV. The accuracy of the results

A. The time factor

Some experiments were run with the same constant gradients for essentially longer periods than half an hour. This was done in an attempt to find out *inter alia* to what extent the response during the course of time

is influenced by adaptation, exhaustion of sense organs, changes in muscular activity, intoxication and/or other possible effects (cf. the experimental diagrams in Figs. 11, 13, and 17). The test fishes used in the experiments are roaches and three-spined sticklebacks, which are representatives of the two main groups with regard to the specific behaviour patterns displayed in the test yard (cf. Chapter IV).

From Fig. 11 it is obvious that the response of the roaches is rather stable during the whole initial gradient period, A1—8, lasting for four hours. A slight trend, however, towards lower reaction values during the later parts of the period is clearly visible.

Keeping in mind that the avoidance reaction in SWL-gradation is due to olfaction (p. 115) this constancy is rather remarkable, as the adaptation of the olfactory receptors might be expected. The most probable explanation lies in the fact that during the continuous movements in the graded environment the roaches are repeatedly confronted with different concentration steps of sulphite waste liquor. This exposes their olfactory organs to changing stimulation. Adaptation is thus counteracted by the renewed rise of the excitability of the sense organs taking place during the visits in the purest mixtures. It is reasonable to assume that the most concentrated mixtures are avoided — which really is indicated in the histograms — after perception of the rising concentration, when the fishes are moving against the gradient. This interpretation is supported by direct observations of the behaviour of the fishes during the experiment. See further discussion in next chapter (p. 80 ff.).

After the cessation of GrA the fishes obviously become disorientated during the immediately subsequent C2. This disorientation is still obvious after the change to GrK, above all during the first 30 minutes, viz. during Kl. Notwithstanding that equally steep gradients prevail — though mutually reversed — the reaction is on the whole less pronounced and less constant during Kl-8 in comparison with A1—8 (cf. also Fig. 10).

It seems reasonable to assume that during the comparatively long stay in GrA the roaches get accustomed to that type of spatially arranged graded environment. The reactions in the corresponding inverse gradient during Kl-8 may be influenced by conditioning established during A1—8. Possible conditioned responses may occur on account of inevitable spatial assymmetries. Other sources of stimuli cannot be disregarded either, notwithstanding the fact that a complete symmetrical arrangement of the test yard has been attempted. Similarly, it was observed by HOAR (1956) and HOUSTON (1957) that young salmon (*Oncorhynchus*) held in confined spaces were able to apprehend and remember spatial arrangements and current.

Another type of after-effect can be seen in the diagram in Fig. 12. It is interesting to observe in the later "controls" (from C13 and onwards) the

turn-overs of the mpv. After cessation of the actual gradients the roaches select the opposite side of the test yard to the parts preferred during the preceding gradient period. The effects on subsequent gradient periods are inconsiderable, however, on account of the strong avoidance reactions shown in the actual steep pH/P_{CO_2} gradients. It is apparent that these reactions are totally overtrumping the disturbing effects due to eventual conditioning, training, or other causes.

The experimental diagram in Fig. 13 for three-spined sticklebacks exhibits a distinct and constant avoidance reaction in not too strong pH/P_{CO_2} gradients of α_1-type during A1—8, i.e. lasting for four hours, as well as during the shorter K1—2. The accuracy of the avoidance reactions of this species in pH/P_{CO_2} gradients is furthermore elucidated in γ_1-gradients as demonstrated in the experiment of Fig. 14. Here GrEF, if sufficiently accentuated, constitutes a barrier which is not passed by the fishes (see EF4 and EF5). If GrAK prevails they gather in the middle sections.

The experiments of Fig. 17 a and b show the reactions of roaches in α_1- and β_1-gradients to dissolved oxygen. The reactions do not appear until about half an hour after the gradients are established. After that the slight positive reactions obtained are quite constant for hours. Consequently an appropriate quantitative estimate of the reaction in an oxygen gradient must be based on mpv obtained after such a state of equilibrium (constancy) is reached.

The experimental results entered into Figs. 11—18 are further discussed in the following chapters.

In this connection they lead to the following general conclusions. (1) Too long test periods are not relevant. Long stay in the same gradient may give rise to training. This in turn influences subsequent reactions. Thus, for example, mpv_{5min} and rv_{5min} obtained sooner or later during an experimental period with the same gradient or a subsequent period with the corresponding inverse gradient are not always quantitatively equivalent. (2) Too short test periods are not representative. (a) A certain variation among rv_{5min} exists. (b) Before the establishment of a complete and fairly constant reaction an initial time of rising reaction often occurs. This is different for different species and test agents. An appropriate experimental procedure must be ascertained in every special case. (3) The procedures employed here would seem to be satisfactory for the present purposes.

B. **Frequency of observations**

The accuracy of mpv and rv must be dependent upon the frequency as well as the number of primary observations. If a certain longer experimental period is divided into a multitude of successive five minute periods, one can expect that the dispersion of mpv_{5min} decreases with increasing frequency

and, consequently, also with increasing number of observations. The problem is to find a frequency at which the dispersion is near its lower limit.

Turning again to the recording film of the experiment of December 18th, 1958 (Fig. 11), this hypothesis is examined in the following way. The primary observations consists of the positions of ten roaches recorded in the sections 1—10 on consecutive frames exposed at 30 second intervals. The mpv_{5min} related to each five minute period covered by ten frames is calculated by utilizing the observation material in five different ways, i.e. corresponding to 30, 60, 150, and 300 second intervals between exposures, respectively. (1) The use of 30 second intervals means that all ten frames are read, and that all mpv_{5min} are thus based upon 100 observations. (2) When dealing with the 60 second intervals the first, third, fifth frame, etc. are taken into consideration, i.e. a total of 50 observations. (3) Concerning the 150 second intervals the first and sixth ones are utilized; altogether 20 observations. (4) Finally, for the 300 second intervals only the first frame, or ten observations are considered. (5) in the last column of Table 8 also, the corresponding values based upon calculations, considering the last (tenth) frame only, in each sequence of ten, is included for the sake of comparison. The difference between the extreme mpv_{5min} of the six ones within each of the 18 successive 30 minute periods (C1, A1—8, C2, K1—8) of the actual experiment is calculated. The range thus observed is put into Table 8 expressed as percentage of the width of the test yard.

Table 8. The difference between the extreme mpv_{5min} (expressed as % of width of trough) within consecutive 30 minute periods in the experiment of Dec. 18, 1958 calculated from different time intervals between exposures.

Test period	Time interval between exposures in seconds and number of observations (in brackets) behind each mpv_{5min}				
	30 (100)	60 (50)	150 (20)	300 (10)	300 (10)
C1	7.3	13.6	17.0	14.0	9.0
A1	4.3	4.8	14.0	17.0	20.0
A2	3.5	3.0	10.5	15.0	21.0
A3	6.6	7.4	9.0	9.0	14.0
A4	5.3	8.4	9.5	9.0	23.0
A5	10.3	13.0	18.0	20.0	23.0
A6	12.4	12.8	18.5	23.0	16.0
A7	3.6	7.0	15.0	18.0	11.0
A8	3.4	10.0	9.5	19.0	19.0
C2	19.8	22.6	17.5	36.0	8.0
K1	13.4	11.8	16.5	25.0	25.0
K2	11.2	11.6	20.5	24.0	20.0
K4	5.2	5.6	12.5	16.0	22.0
K5	15.2	12.2	17.5	18.0	8.0
K6	14.1	15.0	13.0	17.0	22.0
K7	11.8	13.6	22.0	27.0	26.0
K8	10.2	13.6	15.5	15.0	15.0
Mean difference	10.1	10.7	15.4	18.9	17.7

The following conclusions can be drawn from the results in Table 8. The mean difference which is a measure of the variation of the six mpv_{5min} within every 30 minute period diminishes with the increase of the number of records underlying the calculation of mean position value. The mean differences obtained with 30 and 60 second intervals differ very little. On account of this nothing is gained by making exposures more frequently than every 30 seconds. Therefore this frequency of exposures is generally applied throughout the investigation. Anyhow the film recording technique is very tedious.

From the data in Table 8 it is furhermore evident that the range in variation studied does not change considerably with time as long as the same gradient condition prevails. The suddenly increased range from A8 to C2 is obviously related to the change in environmental conditions in the test yard. After the inversion of the gradient conditions the range is high, depending presumably on changed behaviour (cf. p. 58).

If longer experimental periods are used the influence of the frequency of observations on the rv is less noticeable. This appears from Table 9. Different time intervals between exposures, viz. 30, 60, 150, and 300 seconds, as in the case of Table 8, still have some influence upon the rv_{30min} arrived at in the experiment of December 18th, 1958.

It appears from this analysis that the use of time intervals shorter than 60 or 30 seconds does not produce an important increase in accuracy considering rv_{30min} as well as rv_{4hours}.

Summing up the information from the present analysis it is obvious that within certain limits an increased number of observations raises the accuracy of the reaction values. According to Tables 8 and 9 about 50 observations derived from five frames exposed at even intervals seem to be sufficient for a rather good estimate of the "real" reaction, independent of the length of period. Thus less frequent observations are needed, if longer periods are considered. On the other hand — as pointed out on p. 59 — the length of the period is not altogether without importance. A slight trend towards less pronounced reactions during the latter parts of A1—8, for example, is unmistakeable. Thus excessively long periods are misleading. An appropriately shorter period than the actual four hours combined with use of a higher frequency of observations should therefore be chosen. There is no point in choosing time intervals shorter than about 30 seconds (cf. above). It follows that at least 2.5 minute periods are necessary in the actual case of testing ten roaches. In many experiments fewer fish were used. The choice of mpv based on five minute periods seems reasonable to get a good estimate of the preference reaction. Accordingly mpv_{5min} and rv_{5min} satisfactorily follow up the changes in reaction which may or may not be correlated to time or other factors. As can be seen in Table 8 the variation of

Table 9. Rv_{30min} and rv_{4hours} together with the range of rv_{30min} and rv_{4hours} obtained in the experiment of Dec. 18, 1958 calculated on different time intervals between exposures. The table is supplemented by the values of standard deviations based upon the rv calculated in different ways.

	Test period	Time interval between exposures in seconds and number of observations behind each kind of rv_{30min} (in brackets)					Range in % of trough width
		30 (600)	60 (300)	150 (120)	300 (60)	300 (60)	
Rv_{30min}	A1	— 1.95	— 1.92	— 2.11	— 2.05	— 2.25	3.3
	A2	— 1.70	— 1.60	— 1.83	— 1.70	— 1.90	3.0
	A3	— 1.72	— 1.71	— 1.90	— 2.08	— 1.75	3.7
	A4	— 1.67	— 1.68	— 1.44	— 1.50	— 1.50	2.4
	A5	— 1.89	— 1.88	— 1.57	— 1.85	— 2.07	5.0
	A6	— 1.74	— 1.73	— 1.87	— 1.83	— 1.45	4.2
	A7	— 1.52	— 1.41	— 1.54	— 1.83	— 1.63	2.5
	A8	— 1.38	— 1.37	— 1.53	— 1.65	— 1.30	3.5
Rv_{4hours}	A1—8	— 1.70 (4800)	— 1.66 (2400)	— 1.72 (960)	— 1.76 (480)	— 1.73 (480)	1.0
$\frac{s}{\sqrt{n}}$	A1—8	0.06	0.07	0.08	0.09	0.12	
Range in % of trough width	A1—8	5.7	5.5	6.7	7.0	9.5	
Rv_{30min}	K1	+ 0.05	+ 0.11	+ 0.04	— 0.02	— 0.23	3.4
	K2	— 1.00	— 0.95	— 1.13	— 1.15	— 1.22	2.7
	K3	— 0.83	— 0.82	— 1.22	— 1.45	— 0.63	8.3
	K4	— 0.26	— 0.39	— 0.28	— 0.27	— 0.05	3.4
	K5	— 0.98	— 1.02	— 1.02	— 1.02	— 1.03	0.5
	K6	— 0.92	— 0.96	— 1.10	— 0.98	— 0.78	3.2
	K7	— 0.89	— 0.93	— 0.80	— 1.18	— 0.78	4.0
	K8	— 1.16	— 1.11	— 1.15	— 1.22	— 1.33	2.2
Rv_{4hours}	K1—8	— 0.74 (4800)	— 0.75 (2400)	— 0.87 (960)	— 0.93 (480)	— 0.76 (480)	1.9
$\frac{s}{\sqrt{n}}$	K1—8	0.15	0.15	0.16	0.18	0.16	
	K2—8	0.11	0.09	0.12	0.14	0.16	
Range in % of trough width	K1—8	12.1	12.2	12.6	14.3	12.8	
	K2—8	7.0	7.2	9.4	11.8	12.8	

mpv_{5min} within different 30 minute periods in the experiment of December 18th, 1958 usually amounts to about 5—20 % of the breadth of the test yard. On this account it is inadequate to use one or two rv_{5min} on each concentration level. The best method would be to collect a bulk of mpv_{5min}- or rv_{5min}-material from a large number of experiments performed on different occassions and under the same external conditions. Such a procedure would, however, be exceedingly lengthy. In a comparative study with the present purposes experience has shown, that with regard to SWL as well as pH/P_{CO_2} it will be adequate to use the rv_{5min} arrived at in two 30 minute periods of corresponding inversed gradients. However, even fewer than 12 rv will do.

C. The limitations of the statistical treatments

It appears from the descriptions of the technique and procedures employed, as well as of the behaviour displayed by different species (Chapter IV) that the experimental conditions usually are too complicated to make statistical treatments a suitable procedure (cf. e.g. WOOD-GUSH, 1959). This is especially valid for territory holding and/or stationary species. They are all left aside in the following discussion. The attempts at statistical treatments are restricted to species characterized by more or less continuous and arbitrary swimming movements during their stay in the test yard.

Regarding this group as well as the first one mentioned the rv_{5min} are not, however, strictly comparable, as they usually derive from groups of six obtained during 30 minute periods. On this account all rv_{5min} are not independent quantities from a statistical point of view. Nevertheless, it may be plausible to use statistical standard methods for the mere *description* of the results. This is done to some extent as a complement to the graphical presentations. Thereby, to the greatest extent, the statistical information is entered in the pertinent graphs.

The statistical treatment makes no claim to be complete. For example, no attempts are made to work out special mathematical models to fit the basic observation material. This ought to be done, *inter alia,* on account of the fact, that the lateral walls of the test yard constitute barriers for the lateral dispersion of the fishes as well as of the rv. These effects are furthermore complicated as being different for different species (cf. p. 86 ff., Fig. 24).

The momentary visits in the sections, 1—10, of these fish during a certain period, give rise to fairly smooth frequency distributions. Nevertheless, notwithstanding that a constant graded environment prevails, a certain variation occurs among the distributions obtained during consecutive five minute periods. This is examined in the experiment of Dec. 18, 1958. Using the method given by BONNIER and TEDIN (1957, p. 67) the heterogeneity of the six distributions related to every five minute period within every 30 minute period of A1—8 and K1—8 in this experiment is demonstrated in Table 10.

By this procedure the choice of an appropriate length of the test periods will be furthermore elucidated.

In Table 10, χ_a^2 is a measure of the heterogeneity of the six distributions obtained during every five minute period in comparison with the mean distribution calculated on the whole 30 minute period. χ_b^2 is the corresponding value as compared with the mean distribution calculated on the whole A1—8 and K2—8, respectively. K1 is not considered on account of the evidently aberrant reactions during this period (cf. p. 59).

Table 10. Heterogeneity of the six distributions of visits over sections 1—10 recorded during every five minute period within every 30 minute period (A 1—8 and K 1—8) with the same or corresponding inverse gradient in the experiment performed on Dec. 18, 1958.

Test period	f	χ_a^2	P_a	χ_b^2	P_b	Test period	f	χ_a^2	P_a	χ_b^2	P_b
A1	35	18.2	0.99	22.2	0.95	K1	35	60.3	0.004	—	—
A2	35	23.6	0.92	28.7	0.77	K2	40	48.0	>0.1 <0.2	70.7	>0.001 <0.005
A3	35	34.6	0.50	36.6	0.40	K3	40	38.7	>0.5 <0.7	44.4	0.3
A4	35	31.7	0.64	39.0	0.30	K4	35	23.9	0.9	60.3	0.004
A5	35	37.0	0.37	51.5	0.05	K5	40	62.4	>0.01 <0.025	67.0	0.005
A6	35	36.6	0.40	48.8	0.06	K6	40	66.8	0.005	68.6	>0.001 <0.005
A7	35	40.7	0.28	56.7	0.01	K7	40	44.5	>0.2 <0.3	46.1	>0.2 <0.3
A8	35	28.4	0.78	57.1	0.01	K8	40	42.4	>0.3 <0.5	56.8	>0.025 <0.05
Mean		31.4									

Formula used: $\chi^2 = S(\frac{n^2}{m}) - n$; $f = (c-1)\ (r-1)$; (Bonnier and Tedin, 1957; Steel and Torrie, 1960, p. 368).

a = observed number of visits in each of the ten sections during each five minute period.
m = expected numbers of visits in each of the ten sections, i.e., in the case of χ_a^2 and P_a, the mean numbers of the six observations of the number of visits during five minute periods in each section obtained during every 30 minute period and in the case of χ_b^2 and P_b the corresponding mean numbers obtained during A1—8 and K2—8, respectively.
n = frequency sum, here = 100.
f = degrees of freedom
c = number of sections considered.
r = number of distributions considered, here = 6.

The χ_a^2-values indicate increasing heterogeneity from A1 to A3. A3—8 is characterized by rather constant χ_a^2-values, oscillating between 30 and 40. The corresponding P_a-values indicate homogenous "five minute"-distributions all along the line, A1—8. After the change to GrK the χ_a^2-values are with few exceptions higher than during the corresponding inverse GrA. This indicates more heterogenous reactions (cf. p. 58). But with the exceptions of K1 and K6 the "five minute"-distributions within 30 minute test periods are rather homogenous also during K1—8.

Turning to the χ_b^2-values it is obvious that each such value is as a matter of course higher than the corresponding χ_a^2. The rising differences between χ_b^2- and χ_a^2-values during A1—8 from A5 and onwards and especially for A8, must be attributed to the trend toward decreasing reaction values in the later parts of this period. Such a trend is not found in K1—8. Here the highest differences are found in K2 and K4 which indicates that the reaction is not well established.

The conclusions made on p. 62 are supported by the results of this analysis. The choice of 30 minute test periods in advance of longer ones is justified

in the case of the actual experimental conditions, *viz.* roaches and SWL-gradients.

Chi-square may also be used as a purely relative measure for the comparison of distributions of records arrived at under various experimental conditions. Corresponding inversed gradients (cf. p. 25), for example, can be regarded as mutual mirror-images which are nearly but not exactly coinciding (Table 2, Figs. 10, 25 and 28). A similar connection might be expected with regard to the corresponding frequency histograms. This is examined by using the heterogeneity test (Bonnier and Tedin, 1957, p. 67). At first χ^2 is calculated on the basis of the two original distributions, A and K, which are obtained under the corresponding inversed GrA and GrK. The one is regarded as the observed data, the other as the expected ones. This is in the figures denominated "A compared with K". The same procedure is repeated, the only difference being that one distribution is rearranged by exchanging the section numbers in the following manner. Section one is replaced by ten, two by eight, and so on. The latter procedure of calculating χ^2 is called "A compared with inversed K" (see Figs. 10, 25 and 28). The χ^2 obtained in the latter case depends entirely upon factors other than those studied. The difference between the two χ^2-values arrived at is a good measure of the actual reaction.

Finally it will be pointed out. (1) This method of analysing the matter might be very useful in weak gradients giving rise to subtle reactions, i.e., when the establishment of the preference reaction thresholds is the object in view. (2) χ^2-analysis of the present observation material cannot be based uncritically on the significance levels presented e.g. by Fisher and Yates (1953). A correct significance test ought to be based on considerably higher, unknown χ^2-values.

To summarize: the use of conventional statistical methods for the *analysis* and *estimation of the accuracy* of experimental data of this nature is not quite relevant. The accuracy of the present results is clearly demonstrated in the graphs by the dispersions of mpv_{5min} and rv_{5min}. A more penetrating statistical analysis would seem to be quite useless for the conclusions drawn in the present study.

D. The influence of irrelevant external factors on the reproducibility of reaction curves

The influence of external irrelevant factors on the reproducibility of the reactions in successively steeper SWL-gradients is studied with *Phoxinus phoxinus* (Figs. 39, 40, and 41). It appears that the course of the reaction curve is not essentially changed, if (1) different numbers of test fishes are used, i.e. one, three, or eight specimens at a time, (2) if the experiments are performed either in standard illumination (p. 25) or in darkness (records

with the aid of electronic flashes), (3) if the fishes are tested at the flow rate of either 1 cm/sec or 2 cm/sec, (4) if they are tested at different seasons, and finally, (5) if they are studied after being held in the acclimation aquaria for varying periods, which differ by one year or more.

Three-spined stickleback tested in SWL gradients at two different seasons in either case did not show any avoidance or attraction reactions (Fig. 51).

To these results it might be added that (1) a series of experiments with SWL-gradients and roaches coming from the river Dalälven performed in Uppsala water (310 mg HCO_3/l, 8—9° C) and (2) an other series with the same agent and roaches from the river Indalsälven performed in Hölle water (20 mg HCO_3/l, 18—20° C), gave rise to very closely coinciding reaction curves (Figs. 42 and 44). The reasons for this will be discussed more closely in Chapter V. The same α_1-type of combined pH/P_{CO_2} gradiation established at two different flow rates, 1 cm/sec and 2 cm/sec, gave rise to nearly the same rv with roaches tested in Hölle water. Assymetrical light during control conditions supplied by only one of the two tubular lamps in the protective hood above the test yard had no significant effect on the mpv_{30min} on roaches studied in Hölle water. Finally, similar reaction curves are arrived at with combined pH/P_{CO_2} gradients in Uppsala water, if (1) intact roaches, (2) roaches with eliminated olfactory organs, or (3) those with eliminated lateral organs are used as test animals. Experiments with minnows and salmon parr with eliminated lateral organs also give rise to reaction curves which almost coincide with the respective curves for intact specimens.

The main conclusion based on the results of these experiments is as follows. In comparison with the reaction upon the graded environment in itself the other external factors studied are far less important. This supports the statement made on p. 57, that the reaction curves arrived at in the present fluviarium technique are representative for species and agents investigated.

V. **Final discussion**

In the present kind of experimental research work numerous methodological problems are met with. The author is well aware that other ways of procedure might be suggested according to the emphasis which is to be laid upon different specific questions. For the present purpose, however, when a general survey of the responses of a comparatively large number of species, representing different physiological and ecological types, is the object in view, the procedure followed affords a relevant basic material for a conclusive comparative ethological, physiological, and ecological analysis.

Although attempts are made to eliminate all kinds of disturbances, training effects and inequalities as far as acclimatization is concerned certainly

cannot be entirely counteracted. Similarly, certain not easily discernable factors regarding the internal functional state of the test fishes are difficult, not to say impossible, to control.

The quantitative results must be appreciated against the technique employed. The response in the graded environment may have a sensory physiological and/or behavioural background. The acuity of the sensory mechanism is probably not affected by the artificial conditions. The behaviour on the other hand is modified by the confined space of the test yard. The influence of different types of modified behaviour upon the quantitative preference reaction expressed as lateral displacement of mpv from the median line of the test yard will be discussed more closely in the next chapter.

Chapter IV

Some ethological and physiological aspects of the evaluation of the quantitative results arrived at with different species

I. Introduction

Taken directly from their natural habitats and put into the test yard most fishes behave in an excited manner. Importance has been attached to the acclimatization and adjustment to the new artificial environment (p. 29). Thus only fishes appearing to be well adjusted and to a more or less extent tamed are used in the present experiments (cf. DIJKSTRA, 1933, p. 667; and others).

The study of the modified behaviour displayed by the test animals used with the present technique has been concentrated mainly on two species, viz. small specimens of the roach, *Leuciscus rutilus* (L.) and yearlings of the Atlantic salmon, *Salmo salar* L. The standard control conditions [1] are chosen in order to fit the natural demands of these two species as well as possible. The first one is a typically euryoecic species, the second one a more stenoecic fish inhabiting streams with well oxygenated water and low carbon dioxide tension. They represent two ecological types, and behave furthermore in radically different ways in nature as well as in the apparatus. Accordingly, a comparative study of these two species will throw light upon the behaviour as well as the physiological and ecological background of the reaction curves presented in the following chapter. As the main objects of the present investigation they are tested in nearly all types of gradients considered there.

II. Roach

A. The roach as test animal in the fluviarium technique

The roach inhabit the vegetation belts in most shallow lakes and not too swiftly running rivers in Sweden. Smaller specimens are tolerant, easy to secure and handle. They soon become well accustomed to the test yard and are most suitable as test animals in the present technique.

[1] The pike is the only species tested at a somewhat lower flow rate than 1 cm/sec (cf. p. 128), the ide the only being tested at a higher rate, viz. exclusively at 2 cm/sec. The roach was preliminary tested at various flow rates (p. 72). All experimental series with this species considered in the reaction diagrams as well as with other species are throughout the investigation performed at about 1 cm/sec.

B. **Preferred parts of the test area under control conditions**

The schooling behaviour typical for roaches under natural conditions and in big aquaria (SVÄRDSON, 1952; KEENLEYSIDE, 1955; FABRICIUS, 1959) is restrained in the test yard of the fluviarium (LINDAHL and MARCSTRÖM, 1958). The fishes are continually swimming about in the free water, all the time steering up against the current and evidently avoiding touching the bottom or the walls as well as each other. When not meeting with a gradient, they accordingly stay most of the time in the median sections. This is reflected by the usually convex form of the frequency histograms (Figs. 10—12, 15 b, 31, 32, 45 and 46). The distribution shown in Figs. 17 and 25 is an exception from this rule. On comparison to the other ones the specimens considered in the last-mentioned figures are nearly twice as big, however. The roaches are most frequently recorded in the middle zone of the yard (the left histogram in Fig. 22). This does not, however, seem to be quite constant during the course of time as judged from the data arrived at in an analysis of the experiment of December 18th, 1958 (Fig. 19).

The preference for the middle sections during control conditions might be explained by the fact that the current is stronger in the middle than at the sides and bottom. But the variation of flow over the width of the yard is not particularly large. The distribution of the roaches is rather related to the current gradient near the lateral walls or other stimuli deriving from the walls of the confined space (cf. p. 72).

C. **Rheotactic response at different flow rates**

"Fish in a current of water in a confined channel will ordinarily resist being swept downstream and will swim steadily to maintain their position with respect to some fixed reference point, usually detected by sight" (FRY, 1958, p. 93).

The flow rate through the apparatus is first of all defined by the necessary technical conditions, as a certain minimum velocity is required for the complete mixing of test solution with feed water, and as the current must not be too swift, if stable gradients are to be maintained. It is also necessary to regulate the flow as far as possible according to the requirements of the test fishes. A suitable flow velocity should fit as far as possible the natural demand on muscular acticity which occur in the natural habitat. This can probably be achieved within certain tolerable limits. The first criterion is that the roaches during all or most of the experimental time shall stay in the free water passing through the test yard. This is the case from zero up to at least 2 cm/sec (LINDAHL and MARCSTRÖM, 1958) and certainly at still higher rates. The upper limit at which smaller roaches can withstand the current in the fluviarium has not been determined.

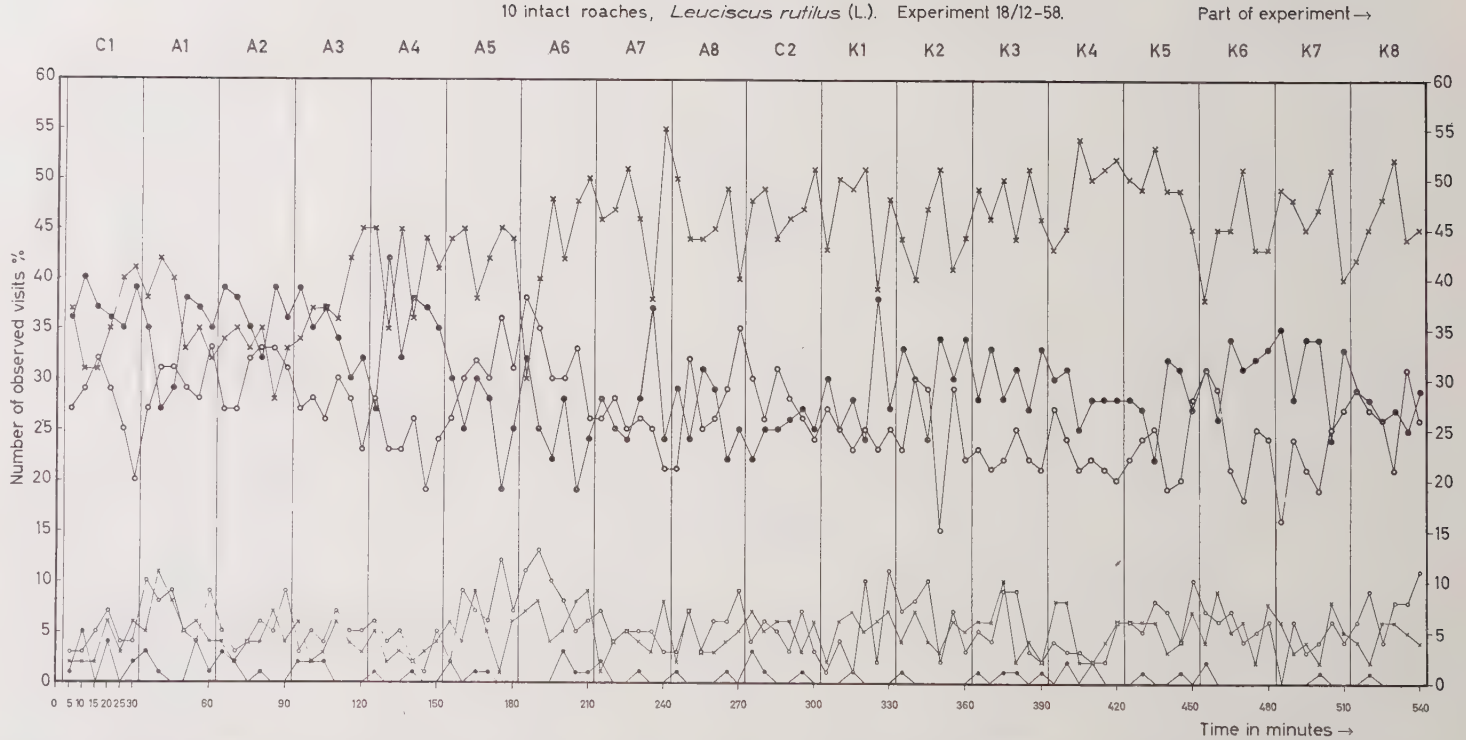

10 intact roaches, Leuciscus rutilus (L.). Experiment 18/12-58.
Part of experiment →
C1 A1 A2 A3 A4 A5 A6 A7 A8 C2 K1 K2 K3 K4 K5 K6 K7 K8
Number of observed visits %
Time in minutes →

Fig. 19. Preferred zones of the test yard (cf. Fig. 1) during the course of time in the experiment of Dec. 18, 1958 (Fig. 11). Observed visits (in per cent of total number) in the front (●) middle (×), and rear (○) zones are calculated for consecutive five-minute periods. Bigger symbols refer to the total amount of observations, smaller ones to the cases when the fishes were recorded as orientated with the current. Cf. p. 71 ff.

The performance of fish in a streaming environment is but incompletely known. The time for the maintenance of swimming against the current in relation to flow rate might, as suggested by FRY (1958), throw light upon the "continuous level of performance that will be terminated by fatigue" (*op. cit.*, p. 93). At present the stress due to fighting against the stream flowing through the fluviarium can not be expressed in physiological terms. Here special problems arise which remain for future work on the active and standard metabolism of fish in connection with locomotor performance. The reader is referred to the investigations by FRY (1947, 1957, 1958), SHEPHARD (1955), BLACK (1958), BRETT (1958), BASU (1959), KATZ, PRITCHARD and WARREN (1959), and HOUSTON (1959).

An increased velocity of flow must be compensated for by intensified muscular activity, if the fish shall withstand the current. This exertion depends upon the velocity of flow as well as upon the size of the fish (HOUSTON, 1959). It might be expected that the energy required for the excess work at higher rates of flow as compared with that at lower ones must result in a reduced frequency of lateral swimming movements. This is also clearly demonstrated in Fig. 20. The preference reaction in a gradient is probably to great extent a consequence of the more or less arbitrary swimming movements of the fishes, especially across the concentration steps created in the test yard (cf. p. 80 ff.). For this reason it seems necessary to define the connection between rheotactic response and velocity of flow.

In order to get the necessary information of a suitable flow velocity a number of preliminary experiments were run. The rheotactic response of 26 small roaches were examined at different flow rates in pure Uppsala tap water. Some experiments with roaches with eliminated lateral organs were also performed (cf. Fig. 20 and Table 11). The positions of the fishes in the test yard were time-lapse photographed in the usual way at 30-second intervals. The rheotactic response at each rate level is determined for every five minute, or longer period, by the examination of every sequence of ten or more frames, according to the following principle: Every record of a fish is compared to a compass needle with the cranial end representing north, when facing the current. Thus the median line of the yard is regarded as north-south axis. The imaginary compass-card round every record is thought to be subdivided (1) by the NE—SW axes into four quadrants or (2) by the W—E axis into two semicircular areas. The numbers of records falling within the different sectors expressed in percentages are calculated for consecutive five minute periods, and put in the diagram of Fig. 20. As seen in this figure and Table 11 the statistically treated observation material indicates a nearly rectilinear relationship between rheotactic orientation and flow velocity within the range from zero to about 1 cm/sec. At the latter flow rate the strength of rheotactic reaction is not noticeable influenced by the two light conditions used, i.e. darkness and standard illumination (p. 25).

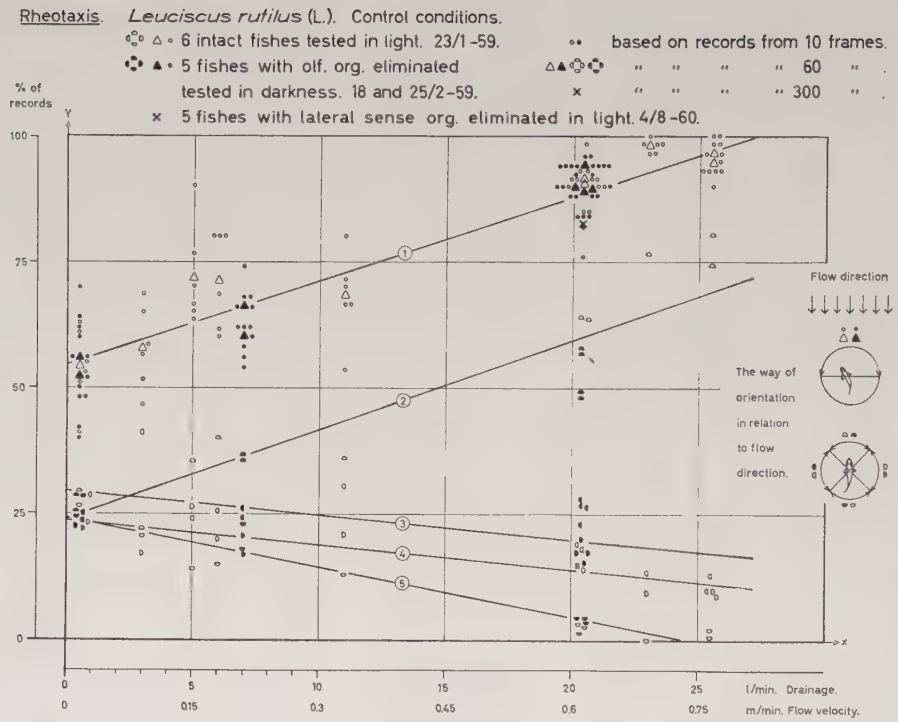

Rheotaxis. Leuciscus rutilus (L.). Control conditions.
6 intact fishes tested in light. 23/1-59.
5 fishes with olf. org. eliminated tested in darkness. 18 and 25/2-59.
5 fishes with lateral sense org. eliminated in light. 4/8-60.
based on records from 10 frames.
" " " " 60 "
" " " " 300 "
% of records
Y
100
75
50
25
0
Flow direction
The way of orientation in relation to flow direction.
0 5 10 15 20 25 l/min. Drainage.
0 0.15 0.3 0.45 0.6 0.75 m/min. Flow velocity.

Fig. 20. Rheotactic orientation of roach in relation to flow velocity through the test yard. Encircled figures signify the centre coordinates of regression lines the equations of which are given in Table 11.

Nor does the reaction of intact roaches differ considerably from the reactions shown by specimens with eliminated olfactory organs or those with eliminated lateral organs.

These results support the observation made by earlier investigators (Lyon, 1904, 1905, 1907; Dijkgraaf, 1933; Gray, 1937; Herter, 1953, p. 44; Hoar, 1954; Bull, 1957) that the "touch response is exceedingly sensitive in orientation to currents in water" (Gerking, 1959 p. 226). According to these authors the positive rheotaxis shown by fishes in a confined space is connected with a negative thigmotactic response. However, I am not quite convinced that the rheotactic response demonstrated here is always released by a direct contact stimulation from the bottom or sides of the test yard. As pointed out above the roaches tend to avoid such contacts. It might just as well be the current gradient near the limiting surfaces, that is perceived, and acts in a similar way as a stimulus.

The displacement of the line No. 3 in relation to the line No. 4 in Fig. 20 and Table 11 can be explained by possible inequalities in stream velocity or other factors. However, the rather good coincidence of the two lines is a consequence of the symmetrically arranged control condition.

D. The space exploitation behaviour

At the standard rate of flow of 1 cm/sec the roaches easily resist the current as can be judged from their tranquil behaviour during control conditions. Under such circumstances plenty of scope apparently still remains for such activities as lateral swimming movements. Easy undulating movements of body and unpaired fin in combination with swift snippings with the pectoral fins characterize their appearance. These movements usually only result in that the fishes remain in the same position, but may also propel them in different directions. Sometimes isolated fishes make runs for longer distances through the test area, and may also swim actively downstream (Figs. 21 and 22). After a longer stay in the apparatus the roaches sometimes tend to arrange themselves parallelly side by side facing the current. Seemingly in a definite order they form a chain stretching over the width of the test yard. Occasionally a fish swept passively backwards suddenly moves on, possibly upon tactile stimulation (cf. p. 72). "Gray, Dykgraaf and Lyon all observed that blinded fish were carried passively downstream by a current until they touched the bottom or sides of the container. Upon contact they immediately orientated themselves with the head upstream." (GERKING, 1959, p. 226). An examination of 10,800 records on the film of the experiment performed on December 18th, 1958, however, does not give any single indication of the fishes coming into bodily contact with the rear net except possibly the most distal ends of their caudal fins.

Anyhow the swimming movements of smaller roaches in the present apparatus imply that all parts of the test yard are continuously criss-crossed in a somewhat arbitrary way. This might be interpreted as a fixed behaviour pattern (TINBERGEN, 1955) released by the artificial conditions in the test yard. It may be called an *exploitation behaviour* which is most pronounced

Table 11. The connection between the rheotactic response of roaches in % as described on p. 71 (Y) and stream velocity in mm/min (x).

Regression lines	Centre coordinates $\bar{x}$	Centre coordinates $\bar{y}$	t^2
1. $Y = 55x + 55.08$ $n = 108;\ r = 0.913$	76.9	395.8	527.97
2. $Y = 60x + 24.05$ $n = 18;\ r = 0.924$	47.8	395.8	104.55
3. $Y = -16x + 29.67$ $n = 18;\ r = -0.560$	23.3	395.8	7.32
4. $Y = -16x + 23.5$ $n = 18;\ r = -0.787$	17.2	395.5	26.02
5. $Y = -33x + 24.16$ $n = 18;\ r = -0.991$	11.1	395.8	877.85

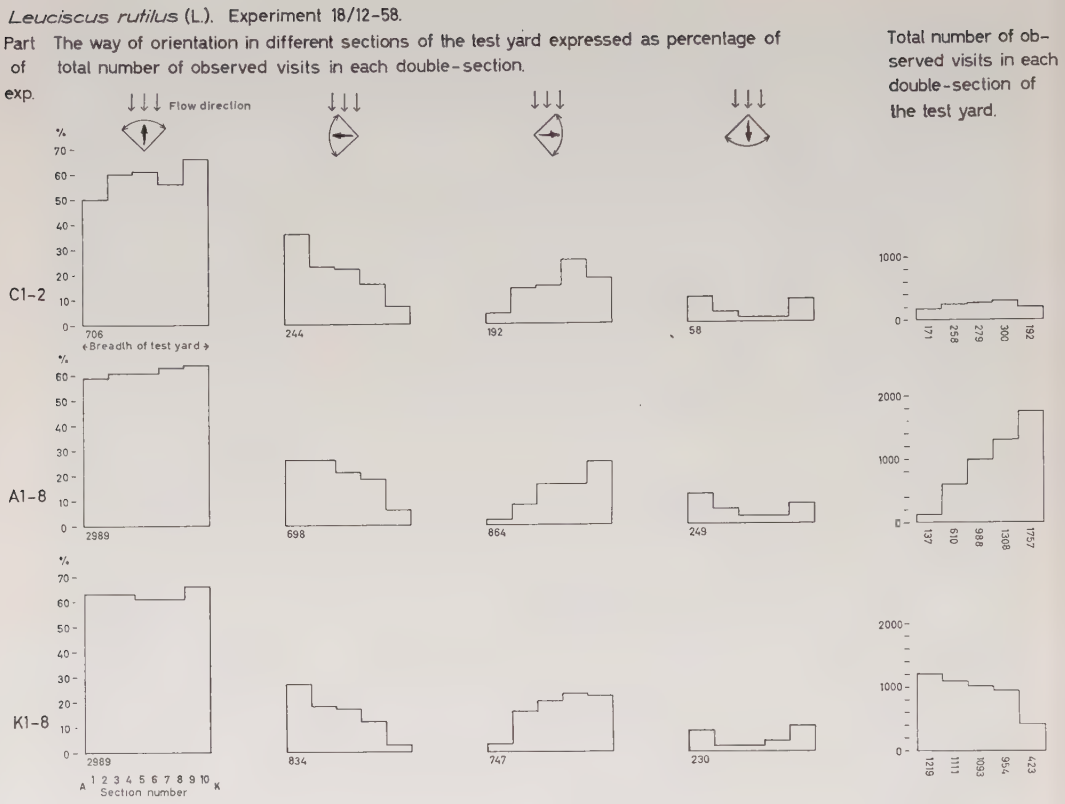


Fig. 21. Rheotactic orientation in different double sections of the test yard during different parts of the experiment of Dec. 18, 1958 (cf. Fig. 11).

and perfectly developed under control conditions. It also constitutes an essential part of the behaviour displayed during different gradient conditions. This principle of "diffusion" is clearly demonstrated in Fig. 12. As seen in this figure the roaches showing pronounced avoidance reactions in the steeper gradients spread rapidly according to the exploitation drive all over the test yard as soon as the gradient disappears (cf. also pp. 58 and 59).

However, this fixed behaviour can occasionally be broken, as for example after heavy external stimulation under adverse environmental conditions. Under such circumstances a more or less transitory school-aggregating response may occur. This behaviour is interpreted as part of an emergency reaction (cf. p. 103). After oxygen depletion in the whole test yard, for example the roaches are found to gather in the central part of the yard. They behave excitedly, and appear clustered at the water's surface (emergency respiration or "Notatmung", cf. Olthof, 1941). Similar reactions are found also at the sudden confrontation with high hydrogen ion and/or carbon dioxide

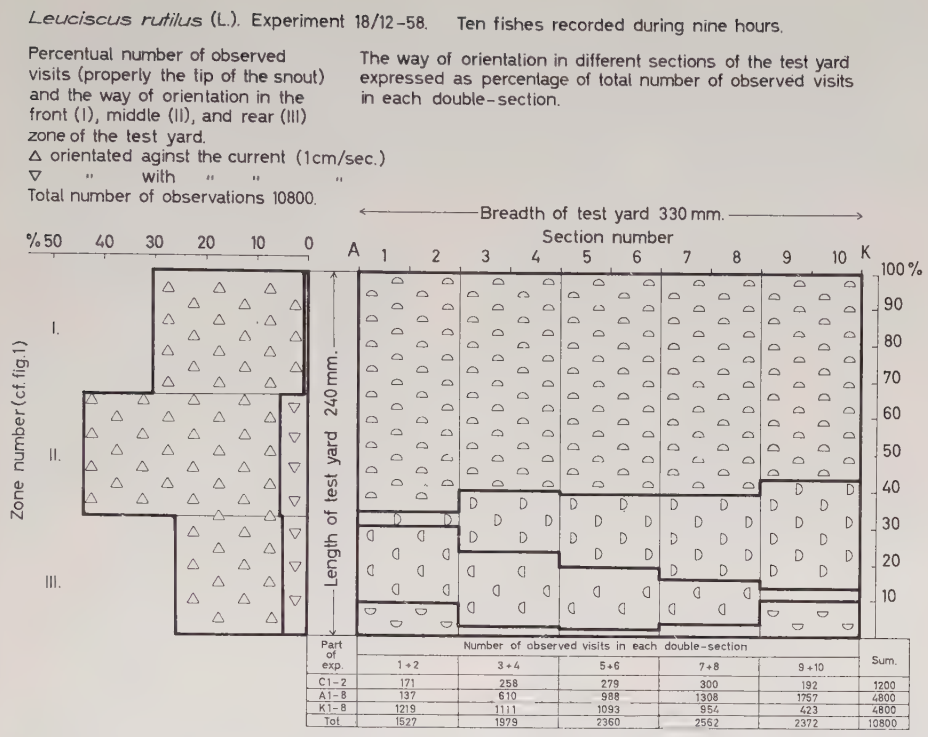

Leuciscus rutilus (L.). Experiment 18/12-58. Ten fishes recorded during nine hours.
Percentual number of observed visits (properly the tip of the snout) and the way of orientation in the front (I), middle (II), and rear (III) zone of the test yard.
△ orientated aginst the current (1cm/sec.)
▽ " with " " "
Total number of observations 10800.
The way of orientation in different sections of the test yard expressed as percentage of total number of observed visits in each double-section.
Breadth of test yard 330 mm.
Section number
A 1 2 3 4 5 6 7 8 9 10 K
%50 40 30 20 10 0
100 % 90 80 70 60 50 40 30 20 10
Zone number (cf. fig. 1)
I.
II.
III.
Length of test yard 240 mm.
Part of exp.
Number of observed visits in each double-section
1+2 3+4 5+6 7+8 9+10
Sum.
C1-2 171 258 279 300 192 1200
A1-8 137 610 988 1308 1757 4800
K1-8 1219 1111 1093 954 423 4800
Tot 1527 1979 2360 2562 2372 10800

Fig. 22. The way or rheotactic orientation in various double sections and zones of the test yard (cf. Fig. 1). All records from the symmetrically arranged experiment of Dec. 18, 1958 (Fig. 11) are considered. For the explanation of the symbols see Fig. 20; cf. also p. 71.

concentrations at the first arrival of the frontier of an advancing, fairly strong concentration gradient. Temporary concentration of specimens belonging to different schooling species of fish in response to external disturbance has been reported (Parr, 1927; Keenleyside, 1955, and others).

E. **Rheotactic orientation in relation to time, confined space, and gradient condition in the experiment of December 18th, 1958**

The preference reactions of roaches in the SWL-gradients used in the experiment of Dec. 18th, 1958 are shown in Fig. 11. As judged from the results presented in Fig. 23 and Table 12 the rheotactic response seems to be very constant throughout all the 108 five minute periods of this experiment. No significant correlation between rheotactic orientation and experimental time is revealed. No significant difference appears in the rheotactic orientation during A1—8, K1—8, and the two control periods of the actual experiment.

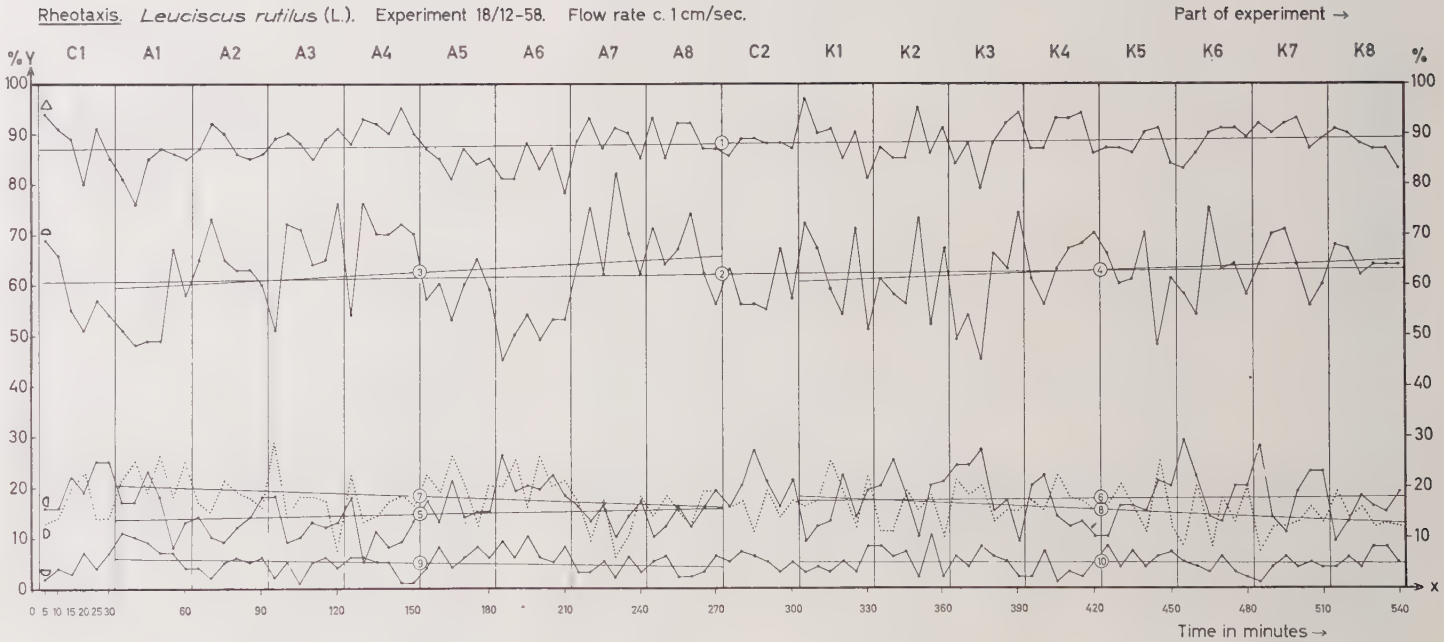


Fig. 23. Rheotactic orientation in relation to time and experimental condition in the experiment of Dec. 18, 1958 (Fig. 11). The symbols entered to the left of every polygonous curve are the same ones as in Fig. 20. Encircled figures signify the centre coordinates of regression lines the equations of which are given in Table 12.

Table 12. The connection between the way of orientation in % (Y) and time in minutes (x) in the experiment with ten roaches performed on December 18th, 1958. Each Y-value is calculated from 100 observations made on ten film frames covering every five minute period. See also Fig. 23.

Test period	The way of orientation (cf. Fig. 20)	Regression lines No.	Equation	r	n	Centre coordinates $\bar{x}$	$\frac{s}{\sqrt{n}}$	$\bar{y}$	t^2	P
C1-K8	△	1	$Y = 0.0041x + 86.76$	0.1675	108	87.90	± 0.37	272.5	3.06	> 0.8
C1-K8	▷	2	$Y = 0.0052x + 60.47$	0.1026	108	61.89	± 0.76	272.5	1.12	≈ 0.3
A1-8	▷	3	$Y = 0.0211x + 59.05$	0.1645	48	62.27	± 1.30	152.5	1.28	≈ 0.2
K1-8	▷	4	$Y = 0.0162x + 55.43$	0.1601	48	62.27	± 1.02	422.5	1.21	≈ 0.2
A1-8	◁	5	$Y = 0.0087x + 13.28$	0.1440	48	14.60	± 0.61	152.5	0.97	≈ 0.3
K1-8	◁	6	$Y = 0.0019x + 16.57$	0.0254	48	17.38	± 0.76	422.5	0.03	> 0.8
A1-8	▽	7	$Y = 0.0215x + 21.28$	−0.3068	48	18.00	± 0.71	152.5	4.78	$< 0.05^* > 0.02^*$
K1-8	▽	8	$Y = 0.0181x + 23.21$	−0.3015	48	15.56	± 0.61	422.5	4.60	$< 0.05^* > 0.02^*$
A1-8	◁ ▷	9	$Y = 0.0085x + 6.48$	−0.2456	48	5.19	± 0.35	152.5	2.95	$< 0.1 > 0.05^*$
K1-8	◁ ▷	10	$Y = 4.79$	−0.0007	48	4.79	± 0.31	422.5	0.023	> 0.9

A1—8 5 compared with 7: $t_k^2 = 0.9425$; $f = 92$; $P \approx 0.3$
K1—8 6 compared with 8: $t_k^2 = 1.3436$; $f = 92$; $0.3 > P > 0.02$

The ethological background of these results are examined more closely in the following way. The problem is to find out, if or to what extent the fundamental behaviour displayed by the roaches under control conditions is changed from one gradient condition to the corresponding inversed one. A possible way of tackling this problem is the examination of the orientations of the fishes as recorded in different parts of the test yard under different environmental conditions.

To start with the following questions arise. How is the orientation of the fishes influenced by the neighbourhood of the walls? How do such possible influences differ during different periods and under various experimental conditions? For answering these questions the frames on the film of the experiment of Dec. 18th, 1958 are examined afresh. This time attention is paid to the distribution over the width of the test yard of the different orientation categories, as defined above (p. 71 (1)). For the sake of simplicity the ten sections are grouped in pairs constituting five double-sections. The numbers of observations belonging to each category are added up from the frames covering the different parts of the experiment. Within each double-section these numbers are expressed as percentages of the total number of observations. The numbers calculated (in %) are arranged as the histograms presented in the first four columns of Fig. 21. In this way the impression of skewness due to the originally skew distributions of all observations during A1—8 and K1—8 is eliminated (cf. Figs. 10 and 11, and the right column in

Fig. 21). The piles of the histograms constructed here actually indicate the frequency of the particular orientation categories. The figure at the bottom left of a histogram gives the total number of observations belonging to the actual category.

Fig. 22 gives a concentrated survey of all the facts obtained by this treatment of the experimental observation material. We can see that the symmetrically arranged experiment gives rise to a fairly symmetrical distribution over the width of the test yard of the different categories of orientation concerned. The fishes being recorded as orientated towards the K-wall are exaggeratedly prominent, since on account of the more pronounced avoidance reaction during A1—8 than during K1—8 the K-side is most frequently visited (Figs. 10 and 21).

The frequency histograms entered into the first four columns of Fig. 21 are essentially uniform within each column. Certain considerable deviations exist, however, above all regarding the middle sections of the histograms showing the frequencies of orientation against the lateral walls (cf. below). The somewhat assymmetrical appearances of the histograms related to C1—2 can be disregarded as being aberrant in consequence of (1) comparatively few observations and (2) disturbing influences during C2 from the preceding A1—8 (cf. p. 58).

The uniformity and symmetry demonstrated in Figs. 21 and 22 give rise first of all to the following suggestions. The frequencies of different kinds of orientation are surprisingly stable within particular double-sections, above all within those situated near the lateral walls of the test yard. This is valid for the whole experiment independent of the quality of the surrounding water. For example during their visits to sections 1 and 2 which are situated nearest the A-wall the roaches orientate themselves so as to face this wall in about 25 % of the recorded cases, irrespective of whether they occur there comparatively seldom (137 observations during A1—8) or more frequently (1219 observations during K1—8). This tendency is a common principle of the experiment. Thus in the sections near the two lateral walls the fishes are throughout the experiment directed towards the nearest wall in about 25 % of the cases, towards the most distant side in less than 5 %, and towards the rear net (i.e. with the current) in about 10 % of the cases. It appears furthermore from Fig. 21 that in more than six out of ten records the roaches all over the width of the test yard face the current in the described way throughout the experiment, maybe to a somewhat less extent during C1—2. As seen in Fig. 22 orientation with the current is more common in the rear parts than further upstream.

This analysis shows a clear tendency on the part of the roaches to face the nearest wall, wherever they are found in the test yard. It is thus obvious that the roaches orientate themselves differently in different parts of the

confined space. Evidently the orientation depends for the greatest part upon influences from the current and the walls, and less from the graded environment.

The next step in the analysis will be to find out to what extent the swimming behaviour as judged from the orientation is changed under existing gradient conditions.

On that account the four histograms which are to be found in Fig. 21 in the second and the third columns, on the second and the third lines are examined. Comparing these histograms mutually and transversely it appears that those placed diagonally are rather good mirror-images of each other. One pair is made up of the histograms related to orientation towards the top concentration side. The other pair consists of the histograms related to orientation towards the pure side of the gradient. The shape of the histograms of the first mentioned pair is more convex than that of the other, due to higher frequencies within the middle sections. This fact is interpreted in the following way. When swimming against the gradient the fishes meet the concentration steps. These influence their orientation in essentially the same way as does the lateral wall on the top concentration side of the test yard. This is typical of A1—8 as well as K1—8. Thus the fundamental behaviour displayed under control conditions is modified in a measure. During test periods with correspondingly inversed gradients this modification follows essentially the same scheme, though in a reversed manner.

The influence of the graded environment upon the swimming behaviour of the fishes is furthermore elucidated by the following considerations. During control conditions the influence of a lateral wall upon the orientation of the fishes decreases as the distance to the wall increases. This can be seen in the second and third histograms on the first line of Fig. 21. The figures at the bottom left of these histograms state that in 192—244 cases out of 1200 during C1—2 (cf. the schedule in Fig. 22) the fishes are recorded as orientated towards one or the other lateral wall. This corresponds on an average to c. 18 % which is taken as a quantitative measure of the genuine effect of one lateral wall upon the orientation of the roaches under control conditions, provided that a rather even and symmetrical distribution of the recorded visits is obtained over the width of the test yard. It is accordingly postulated that under control conditions a skew distribution will give rise to an increased percentage of orientations towards the lateral wall on the preferred side of the yard and correspondingly to a decreased percentage of orientations towards the opposite wall.

In the cases of A1—8 and K1—8 the roaches are recorded as facing the lateral wall on the pure side in not more than $864+834=1698$ (18 %) and as facing the opposite wall in as many as $698+747=1445$ (15 %) cases out

of 9600 primary observations, nevertheless they are definitely recorded more frequently near the wall on the pure side of the gradient.

This analysis leads to similar conclusions as drawn above, viz. (1) the increased percentage anticipated on the pure side is not forthcoming. (2) As the fishes are mainly recorded at comparatively great distances from the lateral wall on the top concentration side the percentage of orientation towards this wall (15 %) is higher than might have been expected. (3) In both cases ((1) and (2)) the effect of the wall on the top concentration side appears to be accentuated, and the effect of the wall on the pure side suppressed. The most probable explanation is, that the rising concentration steps of SWL act as barriers when the fishes are swimming against the gradient, in a similar way, though of course less efficiently, as does the wall on the top concentration side. This interpretation is not contradictory to the fact that the influence of the gradient upon the lateral deviations of the fishes is restricted to the median sections of the test yard.

These findings do not alter the conclusions drawn earlier (p. 79), viz. that the orientation of the roaches is first of all dependent upon the confined space and the current. The characteristic swimming behaviour displayed under control conditions is not essentially changed in moderate SWL-gradients: although it is modified in the middle sections by the concentration steps of SWL acting as directive olfactory stimuli (cf. p. 115). Accordingly the space exploitation behaviour (p. 73) is an essential element in the behaviour under gradient conditions. This is confirmed also by the impression arrived at by direct observations of the fishes.

The orientation reactions found here probably constitute a more common ethological principle, which is valid also for other species and agents. This principle is undoubtedly applicable to weaker and certainly also to stronger SWL-gradients than in this particular experiment. Anyhow it is reasonable to say that the specific swimming behaviour, released in moderate gradients of agents acting as directive stimuli, remains on the whole unaffected for considerably longer periods than 30 minutes, or as long as the fishes are not sensorily adapted.

F. Further discussion on the reactions in gradients

The exploitation behaviour of smaller roaches studied here can be regarded as an "innate" behaviour pattern (Tinbergen, 1955, p. 2) which is specific for the actual artificial environment. Most easily seen under control conditions it originally involves undirected orientation movement or trial-and-error behaviour (Jennings, 1906; Kühn, 1919; Tinbergen, 1955, p. 89) which in the confined space is modified first of all by influences from the current and the surrounding walls.

The rather constant avoidance reaction is perhaps best explained by the supposition of two mutually counter-active principles, viz. on one hand the space exploitation drive and on the other hand directive sensory stimulation being derived from the actual gradient. One can speak about an expansion force (=the space exploitation) and a counter-pressure deriving from the graded environment. There is reason to consider the former component as fairly uniform from one experiment to the other. The intensity of reaction is the result of a kind of equilibrium between the two principles, and thus depends mainly upon the steepness of the gradient as long as the fishes are not physiologically adapted or poisoned.[1] These points of view should be kept in mind, when evaluating the reaction curves presented in this study.

At present I do not intend to approach more closely the delicate problem of the classification of orientation movements. It is difficult to avoid the impression that a good deal of the somewhat confusing discussion of these matters is a battle of words. The co-ordination of the interpretation of the reactions demonstrated here with ideas proposed by earlier workers is, however, complicated, on account of the different techniques and test animals employed. Here I refer to the reviews of FRAENKEL and GUNN (1940, p. 134), TINBERGEN (1955), and THORPE (1956) and even to the classical presentations by JENNINGS (1906), LOEB (1918), KÜHN (1919), UEXKÜLL (1921), and others.

The behaviour underlying the preference reactions of roaches in gradients of a test agent acting as a directive stimulus resembles that of the classic demonstration by JENNINGS (1906) of *phobotaxis* (*sensu* KÜHN, 1919) on *Paramaecium*. Since the side-walls are obstacles to the rectilinear swimming movements it is, however, rather difficult on the whole to decide whether the avoidance reactions demonstrated here shall be attributed to *taxes* or *kineses* (FRAENKEL and GUNN, 1940).

The observations made in the present study may be in agreement with the statement of COLLINS (1952), that the avoidance reactions of *Pomobolus* to *i.a.* CO_2 differences demonstrated by this author are attributed rather to "trial" or klinotaxis than to tropotaxis (*sensu* FRAENKEL and GUNN, 1940). Klinotaxis involves regularly alternating deviations of part or the whole of the body and comparisons of intensities of stimulations, which are *successive in time*. Tropotaxis involves turning away from (or towards) the maximum stimulation by *simultaneous* comparisons of intensities of stimulations perceived by bilaterally and symmetrically arranged pairs of receptors. In the latter case no deviations are required.

[1] Poisoning does not occur in the present study with regard to all species studied, but in a few exceptional cases with very strong gradients of combined pH and carbon dioxide tension and possibly also though less pronounced, in the steepest concentration gradients of sulphite waste liquor (cf. pp. 99 and 122).

The possibility is not excluded, however, that the simultaneous perception of the paired receptors or tropotaxis may play an important rôle in the reactions demonstrated with the present technique. Conclusive arguments for answering the matter in question are still missing. The repetition of the present type of fluviarium experiment after the unilateral extirpation of the olfactory end organs would probably throw more light on this problem. Such experiments are still to be done.

At the present stage of knowledge I incline towards the assumption that the avoidance reactions demonstrated here in gradients of SWL and of combined pH and P_{CO_2} should be considered as a combination of *klino-taxis* and/or *tropo-taxis* on one hand (the directed avoidance, when encountering higher concentration in the swimming about in the test yard above all in the direction against the gradient) and *ortho-kinesis* on the other (the rather haphazard undirected exploitation swimming movements modified by the current and the neighbourhood of the walls).

Along these lines of argument we return to the point of view suggested above. In the cases of (a) SWL and (b) combined pH and P_{CO_2} the preference reactions of roaches can be regarded as the consequence of (1) the space exploitation behaviour (*ortho-kinesis*) and (2) directive stimulation (*klino-taxis* and/or *tropo-taxis*). It should be added that the normal space exploitation behaviour is successively transformed into emergency behaviour at increased adverse environmental conditions. All transitionary states seem to occur. The intensified swimming activity displayed under such circumstances may be undirected. It is more or less temporarily pronounced during the first phases of a gradient period (cf. Fig. 38).

In the case of SWL the directive behaviour is the result of olfactory stimulation (p. 115). This is in close agreement with the essential feature of tropo-taxis, i.e. the orientation movement is released in consequence of the balance between simultaneous comparison of different intensities on either sides of a paired receptor system.

On this acount it is reasonable to assume an analogously paired arrangement of the presumptive receptor system or systems effecting the avoidance reactions demonstrated in combined pH/P_{CO_2}-gradients. As shown later in Chapter V, pH and CO_2 maybe, or maybe not are perceived by different sensory organs. The findings presented in that chapter give evidence for directive reactions to pH as well as CO_2 in the gradient experiments performed. It is not amiss to presume that the underlying receptor systems are associated with paired sensory organs. See further discussion on p. 109.

The reactions in oxygen gradients are not connected with directive stimulation (p. 90). The "attraction" demonstrated in Figs. 17, 25, and 26 is interpreted as the consequence of undirected reactions of the category called *ortho-kinesis* by FRAENKEL and GUNN (1940).

III. Atlantic salmon parr

A. The modified stationary behaviour in the test yard

Among territory holding animals it is a well known principle that one individual suppresses the activities of others, when restricted to a confined space or limited area. This phenomenon is often released by visual stimuli. Among fish it can be observed above all in connection with spawning behaviour (e.g. FABRICIUS, 1951; and others). The juvenile salmonids studied here exhibit a slight inclination to defend territories within the storing aquaria, as well as in the test yard, but far less than in nature, or in the big stream tank at the Hölle Salmon Research Laboratory (LINDROTH, 1954, 1956; KALLEBERG, 1958). On account of the extremely confined area of the test yard a typical territoriality can hardly be developed, when 5—10 specimens are placed together. Aggressive contacts were not observed very often under control conditions in the present fluviarium, but occasionally during the first stay after removal from the storing tank.

After a while the parr measuring 7—10 cm take resting positions on the bottom, at some distance from each other. They remain, on the whole, fixed in their bottom positions as long as they are not disturbed, i.e. as long as control conditions are going on. This is generally valid even for certain gradient conditions, since after an initial preference phase the fishes have taken new positions in the sections conveying well oxygenated and pure water. As was already pointed out on p. 49 this immobile behaviour necessitates the use of two corresponding inversed gradient periods in close succession, in order to obtain clear reactions and correct reaction values especially in weak gradients.

The behaviour of the salmon fry agrees in most details with the description given by KALLEBERG (*op cit.*, p. 60, Fig. 1). "The fish usually lay upon the substratum, facing the current. Upon a smooth surface the pectoral fins were in this case laterally extended and twisted so that their cranial edge was applied to the substratum, while the caudal edge was directed obliquely upwards . . ." An essential deviation exists from the observations made by KALLEBERG in the experimental tank at Hölle. He found that salmon fry measuring about 4 cm which took fixed bottom positions at higher flow rates, left their bottom stations when the velocity of the current was lowered. This changed behaviour was well established at the velocities of 10—5 cm/sec, and at 2—1 cm/sec a very slow upstream migration began. In the pure water streaming through the fluviarium even at the comparatively low flow rate of 1 cm/sec, however, no apparent tendency to leave their bottom stations is found. The most probable explanation is that the confined space of the test yard prevents the parr from moving forward. It shall, however, be added that the stages used in the fluviarium are twice as large as those studied by KALLEBERG.

During the actual artificial conditions the resting positions on the bottom may be regarded as a comparatively fixed and normal behaviour pattern which is a slight modification of that displayed by salmon parr in nature.

B. Reactions in gradients

As will be discussed more closely in the next chapter, the parr left their bottom stations on different external stimulations. Lowered pH, increased carbon dioxide tension, and/or oxygen depletion in the surrounding medium release within certain limits a more or less erratic swimming response (Winterstein, 1908; Reuss, 1909, 1910; Wells, 1913; Tracy, 1925; Dahl, 1926; Dijkstra, 1933; Lindroth, 1947; Jones, 1952; Shephard, 1955, and others). Due to asphyxiation this behaviour may be looked upon as "emergency reactions of the sympathetic nervous system" (Fänge, 1953; cf. p. 103). Diluted sulphite waste liquor does not have a similar effect on the fish used in the present study. This is in agreement with observations made by Williams and others (1953) on young stages of four species belonging to the Genus *Oncorhynchus*.

In the experimental series performed here one has to point out the different behaviour of salmon parr and other species in gradients of the test agents acting as *non-directive* or *directive* stimuli (*releasing* or *directing* stimuli according to Tinbergen, 1955). As will be shown later on in the present study, CO_2, pH, and SWL belong to the latter category, and oxygen deficiency to the former one. The sufficient lowering of pH and the increasing of P_{CO_2} at the same time, or oxygen depletion over the whole test areas, release mutually similar reactions characterized by erratic searching movements — obviously haphazard in nature — all over the yard (Figs. 27 and 38). The same typical searching behaviour released under gradient conditions sooner or later ends with the occupation of new bottom positions in the less intermingled water. This is very obvious immediately after the establishment of pH/P_{CO_2} gradients, but with much less precision in oxygen gradients.

From the ethological point of view the erratic swimming behaviour may be regarded as an *appetitive behaviour* (Craig, 1918; Tinbergen, 1955, p. 104; Houston, 1957, p. 38). The more or less sudden change to stationary behaviour can accordingly be considered as a *consummatory act* appearing on account of failing stimulation. "Holzapfel (1940) has shown that . . . appetitive behaviour may also lead to rest or sleep. . . . rest and sleep are true consummatory actions." (Tinbergen, 1955, p. 106). However, it should be added in this connection that the exhaustion caused by the comparatively vigorous swimming after the releasing stimulation, may just as well accentuate the need for relaxing stationary behaviour (cf. Fig. 27).

In connection with the rearranging movements, agonistic behaviour (Scott and Fredricsson, 1951; Kalleberg, 1958) can occur in a somewhat

higher frequency than usual. In competition for the limited space of the graded environment offering suitable environmental conditions one or two specimens often dominate.

A closer approach to the reactions displayed by salmon parr in combined pH/P_{CO_2} gradients is given in the scheme of Fig. 38 which is further discussed in Chapter V.

IV. **Other species**

With regard to the mobility displayed under control conditions in the test yard, salmon parr and roaches are representatives for the most extreme types which can be discerned among the thirteen species studied with the fluviarium technique. First of all stationary species on one hand and free-swimming ones on the other constitute two main groups.

(1) The following species are more or less stationary when remaining in the test yard. (*a*) Some test fishes stay most of the time on the bottom, e.g. parr stages of the Atlantic salmon and of the brown trout. Other juvenile salmonids like the char and the brook trout behave similarly, but intermingle by more or less swimming in the open water. Similar differences in behaviour among different species of juvenile salmonids are commonly observed also in the rearing troughs by people at fishery plants. (*b*) Other species appear mainly stationary in the free water. The three-spined stickleback and young of the pike belong to this group. Facing the current steadily twisting their pectoral fins and undulatorily moving their unpaired fins these species remain in position.

(2) Secondly, there are fish which all the time or most of the time move about, criss-crossing all parts of the test yard. (a) The roach, the ide, and the whitefish (*Coregonus nasus*) are mainly staying in the open water. (b) The minnow, young of the tench, and perch fry are mainly swimming in the open water or along the bottom upon which they more or less occasionally may take a short rest. (c) Finally, specimens of the crayfish continually crawl about, exclusively upon the bottom.

Studied under control conditions different species also behave differently with respect to their preferences for different parts of the test area. These matters will be explained by the following exposition.

(1) Some species most frequently visit the sections nearest the lateral walls. The crayfish show a decided preference for the corners and the lateral sections of the test yard. Perch fry (about 3 cm long, cf. Fig. 19) generally swim with vigorous body undulations from one side to the other, mainly along the rear net. Coming near a lateral wall they hesitate, rest for a while on the bottom, or turn back again at once. Under gradient conditions the concentration steps influence this behaviour, especially when swimming against the gradient.

Taken directly from their natural habitats minnows behave more excitedly in the test yard than do other species. After becoming tamed the schooling instinct of the minnow seems not to be inhibited to the same extent as in the case of the roach (p. 69). Swimming about in the test yard the minnows frequently stay near the walls and in the corners (Figs. 7 and 8). This tendency seems to totally disappear after the extirpation of the lateral organs (Fig. 15 a).

Among the stationary species the three-spined stickleback shows a clear predilection for wall proximity (Figs. 13 and 14).

(2) Other species appear to avoid coming near the walls of the confined space. With some exceptions (Figs. 17 and 25) this is usually valid for the roach (Figs. 10, 15 b, 31, 32, 45, and 46). The same seems to be characteristic for minnows with extirpated lateral organs (Fig. 15 a).

(3) The remaining species studied under control conditions are characterized by a more even distribution of the recorded visits over the width of the test yard. This is for example demonstrated for the ide in Fig. 9, for parr stages of the Atlantic salmon in Figs. 33 and 34, and also for the roach in Figs. 17 and 25.

V. **Final discussion**

It appears from the preceding considerations that under gradient conditions the distributions of the fish in the test yard depend on (1) the concentration steps (2) the specific behaviour displayed by different species in relation to the confined space.

The numerical reaction values used are defined (p. 48) as the lateral deviation of the average position of an actual frequency distribution from the median line of the test yard. The rv found in this study do not really express the reaction to the gradient itself. Mobility and predilection for wall proximity are of greatest importance in the evaluation of the reactions obtained. The following discussion will be restricted to avoidance, on the assumption that no adaptation or intoxication occur. The purpose is to outline the essential connections between the rv actually obtained, (in Fig. 24 called observed rv) and the real responses expected to be merely due to the graded environment (in Fig. 24 called expected rv).

Discussing particular curves suggested in Fig. 24 we start from the fairly plausible generalization that a free-swimming appearance characterized by equally frequent visits in all the ten sections, gives rise to a direct proportionality between observed and expected reaction values, as long as the gradients are not too steep. This is demonstrated by the curve D. The ide and the whitefish in particular, but also the roach (curve E) and the minnow, behave fairly well in accordance with such an ideal pattern.

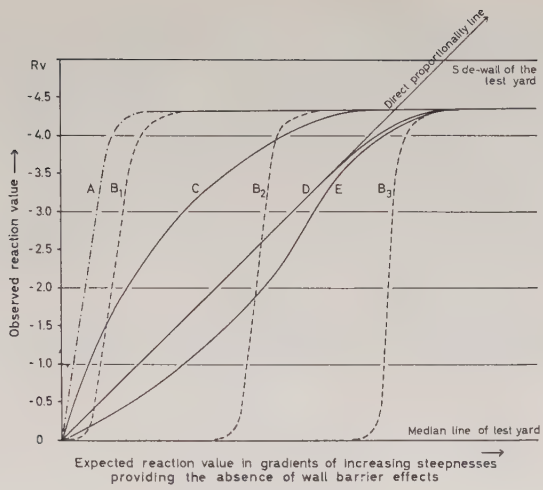


Fig. 24. Hypothetical scheme of the essential differences in the effects of the confined space which might be expected upon the observed rv on account of specifically diverse behaviour patterns. A, Species with stations in the open water and predilection for wall proximity, e.g. *Gasterosteus aculeatus*. B, Species with more (B_2 and B_3; e.g. parr of *Salmo salar* and *S. trutta*) and less (B_1; e.g. parr of *S. alpinus* and *S. fontinalis*) fixed stations on the bottom and a certain predilection for wall proximity. C, Free-moving species which prefer to stay near the lateral walls, e.g. young of *Perca fluviatilis*. D, Free-moving species which are more evenly recorded all over the width of the test yard, e.g. *Leuciscus idbarus*. E, Free-moving species which tend to avoid the lateral sections, e.g. *Leuciscus rutilus*.

Accepting this way of putting the matter it appears furthermore, that species which show some predilection for wall proximity will give rise to numerically too high observed rv, above all in weaker gradients. This is valid for the three-spined stickleback (curve A) and the perch fry (curve C). See also Figs. 13, 14, and 19, in which a preponderant occurrence is found along the lateral wall on the pure side of a gradient. This effect is most pronounced with regard to stationary species (curve A). On account of its stay in the open water the three-spined stickleback might be expected to change position upon external stimulation more readily than species taking resting positions on the bottom, like the juvenile salmonids which are represented in Fig. 24 by the hypothetical curves, B_1, B_2, and B_3. The position of B_1 which has been suggested as representative of the brook trout draws attention to the fact that this species is less fixed to the bottom than other juvenile salmonids. If the change from fixed bottom positions to free-swimming appetitive behaviour needs heavier stimulation, which presumably is the case with regard to the parr of the Atlantic salmon and the brown trout, the whole curve must be thought to be displaced some distance in the direction against higher expected rv. Three possible incipient stimulation levels for releasing the appetitive behaviour are thus exemplified by the hypothetical positions of the curves B_1, B_2, and B_3.

At last it should be emphasized that the roughly hypothetical representation suggested in Fig. 24 is not valid as far as quantitative relationships are concerned. It is merely considered as a hint to be cautious when comparing the reaction curves arrived at with different species. The complexicity of relationships between stimulus and response, applied on methodological problems in pharmacology, has been elucidated by LOEWE (1959). At the consultation of this article a still more complicated interrelationship attached to the present experimental data is obvious.

According to the definition employed here (p. 48) numerically higher rv than −4.5 (corresponding to the median line of the outmost section on the pure side of the gradient) cannot be obtained even if very strong gradients are used in the experiments. This is indicated in Fig. 24 by the gradually retarding slopes in the upper ranges of the curves suggested. That means that all observed rv near −4.5 are numerically too low in comparison with the expected ones. The retarding effect of the wall barrier upon the observed rv is comparatively greater with regard to the free-moving species than in the cases of more stationary ones.

Chapter V

Preference reactions in gradients of various steepnesses

I. Reactions to oxygen

A. Background

One of the most common detrimental effects of domestic and industrial pollution upon fish life is oxygen depletion. It may appear within restricted areas on account of the bio-oxidative decomposition of discharged organic matter. Adverse respiratory conditions may also occur in less contaminated inland waters, especially in connection with the summer and winter stagnations.

The demands of fish for oxygen and other environmental factors interfering with respiration have been studied repeatedly, but have not yet been adequately investigated (cf. Black, 1951; Doudoroff, 1957, p. 413; Fry, 1957; Davidson, *et al.,* 1959; and others). The ability of aquatic animals to detect and avoid areas with deficient oxygen has been studied more rarely. Diverse experimental techniques (cf. p. 12) have been applied to different freshwater fish (Shelford and Allee, 1913; Shelford and Powers, 1915; Wells, 1915; Olthof, 1941; Jones, 1952; Whitmore, Warren, and Doudoroff, 1960). These studies are not all quite comparable (cf. pp. 12 and 95) and rather divergent conclusions have been proposed by different authors. Thus, as pointed out recently by Whitmore *et al.* (1960), "much remains to be learned about the nature of the reactions . . . and the extent to which different oxygen concentrations are avoided by various fish species" (*op. cit.* p. 17). Complementary studies in the laboratory and in the field are therefore needed.

B. Experiments and results

The experiments with oxygen gradients performed in the present study are restricted to three species, viz. roach, Atlantic salmon parr, and crayfish. The data of different experimental series are to be found in Table 1 (p. 12). The results are presented in Figs. 17 and 25—28.

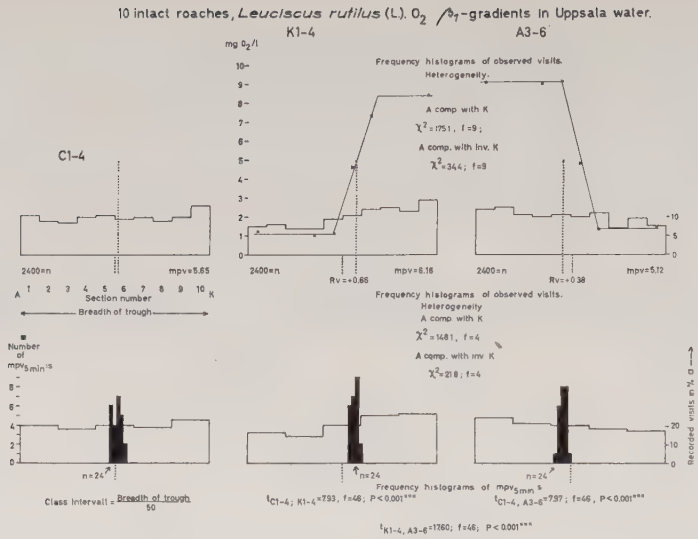


Fig. 25. Preferred sections (upper frequency histograms; cf. p. 46) and preferred double sections (lower ones) of roach in pure Uppsala water (C1—4) and in corresponding inversed β_1-gradients of O_2 (lines and crosses at K1—4 and A3—6). Black areas show the distributions of mpv_{5min}. For the explanation of the statistics see the text on pp. 65. Cf. also Figs. 17 and 26.

C. **Discussion**

1. *Roach*

Judging from the present observations, *oxygen* is a *non-directive stimulus,*[1] that is, deficient oxygen acts merely as a *releasing stimulus*[1] which causes an increased swimming activity (emergency reaction; cf. p. 94). The positive reactions (p. 49) shown in Figs. 17, 25, and 26 are due to *ortho-kinesis* (FRAENKEL and GUNN, 1940; cf. also p. 82). This interpretation implies that the fish only show random movements. They are recorded more frequently in the higher concentrations of oxygen gradients exclusively on account of the fact that they behave more tranquilly there than in sections poorer in oxygen.

The statements made above are supported by the following facts. (1) Direct observations of roaches passing the steep concentration step of a β_1 gradient of oxygen (Figs. 17 *b*, 25, and 26) give no evidence of the instantaneous perception of this substance. The fish pass from the higher range to the lower without any hesitation. (2) There is a comparatively long refractory time (about half an hour) before a fairly constant attraction is established (A1 in Fig. 17 *a* and *b*). (3) No essentially different reactions are obtained in α_1 and β_1 gradients (Figs. 17 *a* and *b*). (4) Gradually decreasing reactions

[1] The terms are used in the sense proposed by TINBERGEN (1955).

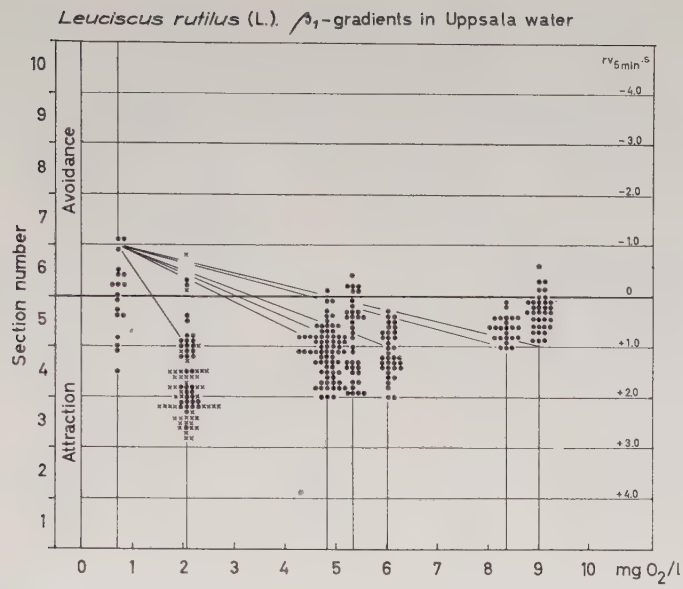


Fig. 26. Rv_{5min} (dots and crosses) plotted against top concentration of steeper and steeper β_1-gradients of O_2, which all exhibited the same low concentration level (at about 0.8 mg O_2/l) on one side of the test yard. Crosses and dots represent fish previously acclimatized to various oxygen regimes (p. 91).

are obtained in a series of β_1 gradients with constant lower oxygen level and with gradually increasing upper ones (Fig. 26). This cannot be explained by irrelevant experimental conditions. CO_2, for example, is driven out most efficiently in the case of high oxygen content. If detectable CO_2 gradients had arisen this way, the fishes must have been affected quite contrarily to the reactions obtained (cf. section II of this chapter). (5) As demonstrated in Fig. 26, roaches acclimatized to a low oxygen regime, viz. about 0.8 mg/l for 90 minutes (crosses), show more prompt attraction in the same β_1 gradient (upper level 2 mg/l) than do those held *ceteris paribus* at about 9 mg/l (dots) ($t=2.96$; $f=82$; $0.01^{**} > P > 0.001^{***}$).

These results must be considered against the background of possible connections between behaviour and the need of environmental oxygen. In β_1 gradients with top levels apparently well above the actual critical tension [1] (Figs. 17 *b*, 25, and on the right of 26) the exploitation behaviour (cf. p. 73 and C 1, C 2, etc. in Figs. 17 *a* and *b*) remains largely unchanged. This means that the respiratory distress which might be expected during the stay in the parts of the test yard with low oxygen concentration does not find expression. It is obviously compensated during the visits to the opposite side, either by excess oxygenation or by a rapid reparation of an induced oxygen

[1] The critical tension is defined as the lowest concentration of oxygen which does not lower the oxygen consumption of the animal (LINDROTH, 1940).

debt. Changes of pH, bicarbonate, and gas content in the blood, necessary to provoke an emergency reaction, are presumably not induced to the same extent as in gradients with the upper level near or below the critical oxygen tension (Fig. 26). In the latter case emergent behaviour is released more or less instantaneously after entering the low concentration. The intensity is probably related to the degree of respiratory distress which may gradually be extinguished by increased ventilation. To what extent the latter is controlled from the respiratory centre or by reflexes upon the stimulation of external receptors in the gill region is unknown.

The presumptive reaction curve which can be drawn on the basis of the rv_{5min} presented in Fig. 26 seems to be fairly continuous. No discontinuity can be discerned which might be connected with the actual (not exactly known) critical oxygen level. Too hasty conclusions should not be drawn, however, from this indication. For example, the following complications have not been clearly surveyed at the present stage of information, (1) The swimming activity may gradually be depressed at lower oxygen concentrations (cf. GRAHAM, 1949; GIBSON and FRY, 1954; FRY, 1957). KATZ, PRITCHARD, and WARREN (1959), however, report a surprisingly high ability of juvenile chinook and coho salmon to swim actively for several hours against a current at oxygen concentrations close to the minimum tolerable level. (2) In β_1-gradients with the upper level near or below the critical tension, increased swimming activities may be induced at both levels.

2. *Parr stages of the Atlantic salmon*

More preliminary tests in β_1-gradients of oxygen showed that salmon parr leave their bottom positions and show a typically erratic swimming behaviour (Fig. 27). They show no abrupt reaction at the passage of the steep concentration threshold in the median part, nor any immediate preference for either side of the test yard. New bottom positions are gradually taken up in the parts of the yard conveying well-oxygenated water. In this way a positive reaction (cf. the definition on p. 49) is obtained after a fairly long "refractory" period. This can be explained, however, without the presupposition of any immediate directive influence of oxygen.

3. *Crayfish*

Also the strong positive reactions of crayfish (Fig. 28) can be explained as the result of ortho-kinetical movements which, unlike those of the roach, are accompanied by a strong predilection for wall proximity (p. 85). The latter is demonstrated by high frequency in the outermost section on the top concentration side. The same tendency also appears, though less pronouncedly, along the opposite wall. The strong attraction of crayfish is also explained by the high critical oxygen tension of this species. According to

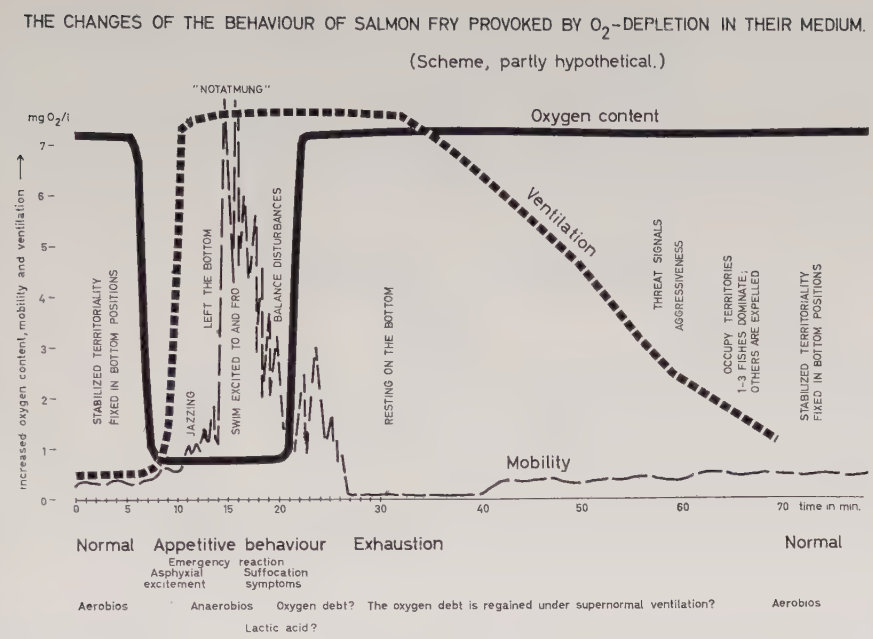


Fig. 27. The essential reactions of salmon parr released at 15 minutes' oxygen depletion all over the test yard. O_2, ventilation and mobility are visualized as plotted against time. O_2 is analysed (acc. to Winkler) and given as stated by the left-hand vertical scale. Ventilation and mobility are roughly estimated and given according to arbitrary ordinate scales.

Lindroth (1937, 1940, 1950) it is, at rest and 15°C, 80 mm Hg or about 5 mg/l for the crayfish, in comparison with 20—25 mm Hg for the roach. Both species belong to the group of test animals which under control conditions continually move about in the test yard. In both cases the mobility is intensified at low oxygen content. Lindroth (1950) studied the reactions of crayfish to low oxygen pressure. According to this author, "a slight tendency towards air seeking" began at about 1.5 mg O_2/l when the temperature was 10°C, and at 1 mg/l the animals showed still livelier reactions.

4. *Concluding remarks*

Though a clear attraction was obtained in the present experiments a non-directive influence of oxygen upon the reactions is supposed in all three species tested. The idea of the nature of the attraction arrived at in the present study is based on the effects of the emergency reaction provoked on respiratory distress. This will soon pass off, providing that the animals have an opportunity to reach an environment with sufficient oxygen (cf. Fig. 27). If not, the fish will succumb sooner or later because, with some exceptions (cf. Blazka, 1958), "there is no evidence for extensive anaero-

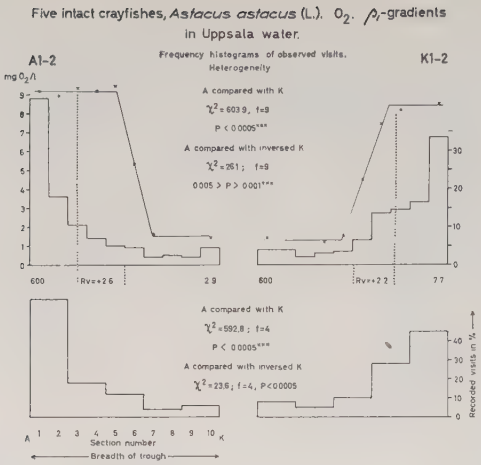


Fig. 28. Preferred sections of crayfish in β_1-gradients of O_2. Cf. Fig. 25. For the explanations of the statistics see p. 65.

biosis in fish" (FRY, 1957, p. 37). The intensity of attraction is due to the combination of increased mobility (released via the respiratory centre?) and preference for staying near or apart from the walls in the confined test yard.

Neither has any convincing evidence been gained by earlier writers that oxygen acts as a directive stimulus. The present explanation is not contradictory of the early statement by SHELFORD and ALLEE (1913, p. 261) that "fishes react to oxygen gradient though usually indefinitely". It seems to be in agreement with the results and conclusions presented by JONES (1952) regarding *Gasterosteus aculeatus, Phoxinus phoxinus,* and *Salmo trutta* and by COLLINS (1952) regarding two species of the genus *Pomolobus.* It does not support the statement of WHITMORE and others (1960) that there are indications of "prompt directonal changes of movements of fish which had entered channels with oxygen deficient water" (*op. cit.,* p. 25).

The driving force behind the attractions in oxygen gradients is an emergency behaviour caused by incipient suffocation. This behaviour, appositely called by LINDROTH (1947) "the restlessness of respiratory distress", can also be considered as an *appetitive behaviour.* This is most clearly distinguished in species with a stationary behaviour in the test yard, viz. in the salmon parr (Fig. 27). Similarly, the increased activity of metamorphosing salmonids during the stay in freshwater, resulting in downstream migration, is designated an appetitive behaviour by HOUSTON (1957). It is followed by a decreased activity after the entrance into salt-water (HUNTSMAN and HOAR, 1939; SHEPHARD, 1948; HOAR 1953; HOUSTON, 1957, 1959). This changed behaviour is hypothetically correlated with changes in thyroid

activity and different osmoregulatory stresses in the two media. Whether it "is due to some specific action on the neuromotor system or to a general change in behaviour is unkown" (HOUSTON, 1957). Much remains to be done until the reactions shown in oxygen as well as in salinity gradients are fully understood from the physiological and ethological points of view.

III. Reactions to pH and CO_2

A. Background

Evidence of the ability of fish to detect carbon dioxide and avoid the higher carbon dioxide concentrations of a gradient was first presented by SHELFORD and ALLEE (1913), who introduced the gradient tank method (cf. p. 12). Using this technique SHELFORD and POWERS (1915) found that *Clupea pallasi* showed sharply negative reactions to CO_2 and H_2S. The explanation that "fish are as sensitive to acidity as lithmus paper" (*op. cit.*, p. 331) and other statements by students working with the gradient tank (e.g. WELLS, 1915 *a*; HALL, 1925) are misleading or based on incomplete observations. The effects of carbon dioxide and pH are not clearly distinguished. On the whole, as also pointed out by JONES (1948), much can be objected to in these early works. For exemple, the dubious method of driving fairly immobile test fish from one part to the other of the tank was practised by SHELFORD and ALLEE (1913, p. 231) and WELLS (1915 *a*, p. 224).

A great deal of work was later done by POWERS and co-workers in an effort to trace the influence of environmental carbon dioxide (and pH) on the orientation and general physiology of fish (e.g. POWERS, 1921, 1922, 1930, 1934, 1937, 1939, 1941, and 1943; POWERS and HICKMAN, 1928; POWERS, ROSTORFER, SHIPE, and ROSTORFER, 1938; POWERS, SHIELDS, and HICKMAN, 1939; POWERS and CLARK, 1942 and 1943). Some of the information and conclusions presented in these studies are, however, disputable (cf. BAKER, 1942, DOUDOROFF and KATZ, 1950; DOUDOROFF, 1957). Using the present technique it was impossible, furthermore, to verify some results obtained with the gradient tank by POWERS and CLARK (1943) (cf. p. 109).

More recently, other techniques have been used in the study of the reactions of fish to pH and carbon dioxide. Given a choice in a narrow horizontal tube (see p. 12; and JONES, 1947) between tap water (pH 6.8) and an alternative water (pH 3.2—12.0) to which HCl or NoOH had been added, JONES (1948) found that *Gasterosteus aculeatus* avoided water more acid than 5.6 or more alkaline than pH 11.4.

In a two-channelled trough (p. 13) submerged in the stream COLLINS (1952) presented two species of the genus *Pomolobus* with a choice between two water qualities during their upstream migration to the spawning areas. The fish selected warmer water (threshold difference 0.5°C) and water of lower

free CO_2 (threshold difference 0.3 ppm or 0.1 mm Hg when the CO_2 content varied from 0.8 to 7.1 ppm). This very delicate response to CO_2 was obtained in well-oxygenated water. As pointed out by CHIDESTER (1922), with reference to his own experience and the experiments performed by SHELFORD and POWERS (1915), subtle reactions are shown also by other members of the family *Clupeidae*. COLLINS (1952) draws further attention to the suggestion by POWERS (1939) that high sensitivity to carbon dioxide may be correlated with changed metabolism in connection with the fasting and development of the gonads of the migrating fish. However, no evidence seems to exist for this interesting idea.

Like POWERS (1930) and JONES (1948), COLLINS (1952) also arrived at the opinion that pH is largely ineffective as a directive factor. A more comprehensive study, however, of the extent to which the avoidance of different species is influenced by CO_2 or by the accompanying difference in pH in combined pH and P_{CO_2} gradients is still lacking. It is one of the purposes of the present investigation to throw more light upon this special question. This is furthermore necessary for the full understanding of the preference reactions shown in a series of experiments with successively steeper gradients of SWL which will be dealt with more closely later on in this chapter.

B. **Experiments and results**

The orientative effects of pH and CO_2 tension upon various fish are here studied in two types of combined pH/P_{CO_2} gradients. The theoretical premisses and the chemical definitions of the solutions in the test yard are dealt with in Chapter II. Thus, using Uppsala water with a high bicarbonate content (c. 310 mg/l) or Hölle water with less bicarbonate (c. 20 mg/l), steeper and steeper gradients up to very steep ones are obtained by the addition of increasing amounts of HCl or NaOH. With reference to Fig. 16 it can be stated at once that the effects of Na^+ and Cl^- ions added this way have no directive influence upon the reactions of the fish in the present experiments.

The species chosen as test fish (Table 1) represent different ecological and physiological types which are characterized by different sensitivity[1] or resistance to dissolved gases in the environment which may interfere with their respiratory functions. The fishes also represent various types from the ethological point of view (cf. Chapter IV). Different aspects of the results are presented in Figs. 12—16 and 29—38.

Two kinds of reaction diagrams are used.

(1) The reaction diagrams of Figs. 29 and 30 are constructed as described below. A similar procedure is also applied in Table 13 and Figs. 31—34. On

[1] CO_2 *sensitivity* may be defined as the asphyxiation level of oxygen at various levels of CO_2 (HART, 1957). Cf. the footnote on p. 106.

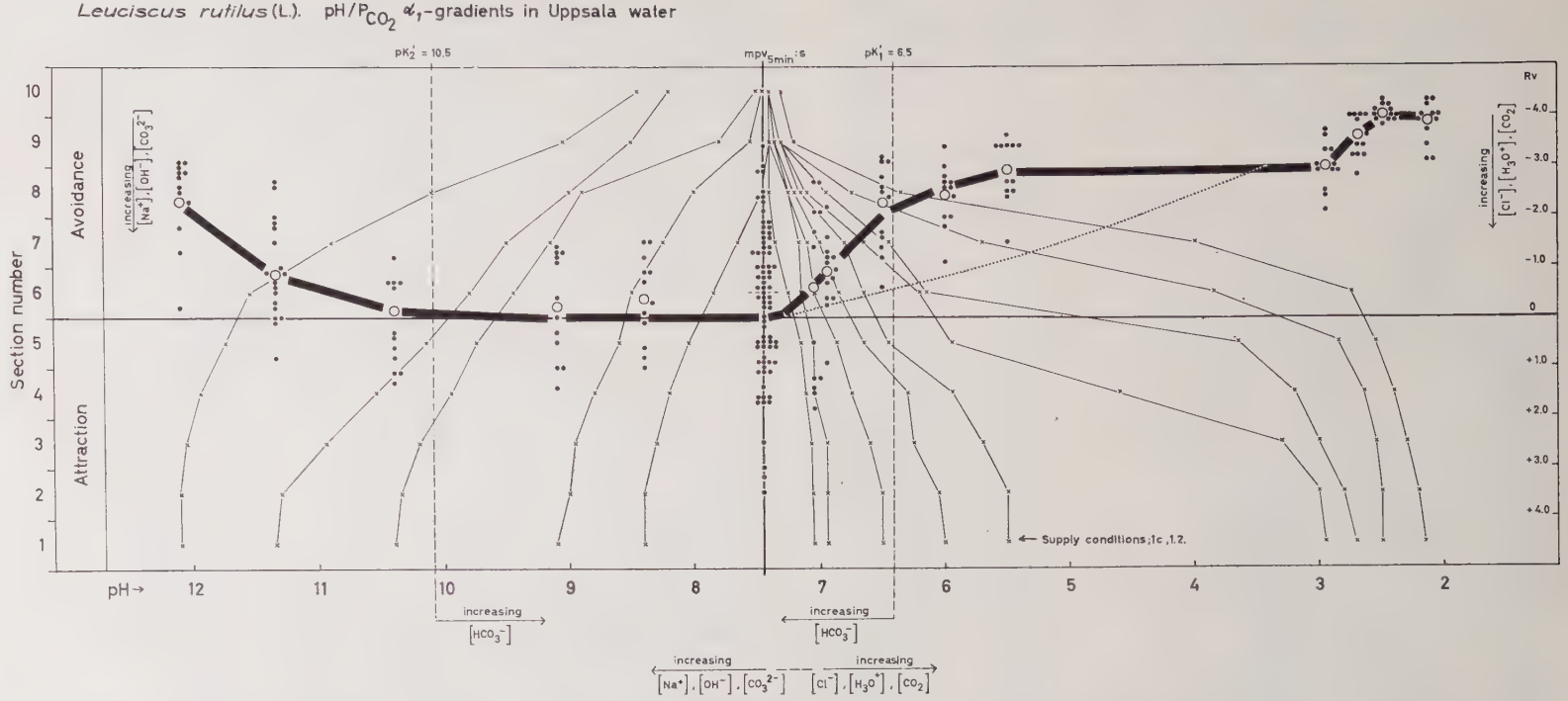


Fig. 29. Reaction diagram (cf. p. 56) showing the preferences of roach in α_1-gradients of pH/P_{CO_2} of varying steepness established in Uppsala water by the addition of various amounts of NaOH (to the left in the graph) or HCl (to the right). The reaction curve (heavy one) is based on 78 mpv_{5min} (dots on the median vertical line representing pure water all over the width of the test yard) from 13 control experiments lasting for 30 minutes, and 168 rv_{5min} (dots) or 14 rv_{60min} (circles) obtained in 14 pairs of corresponding, inverse gradients (cf. Fig. 12). The actual gradients are visualized by crosses (based on pH measurements in the ten sections) connected by thin, straight lines. Rv obtained in particular gradients are plotted against the pH on the top concentration side. The curve of short dashes is the reaction curve arrived at in approximately the same pH gradients established in Hölle water (see Fig. 30). Cf. also Figs. 31, 32, 36, and 37.

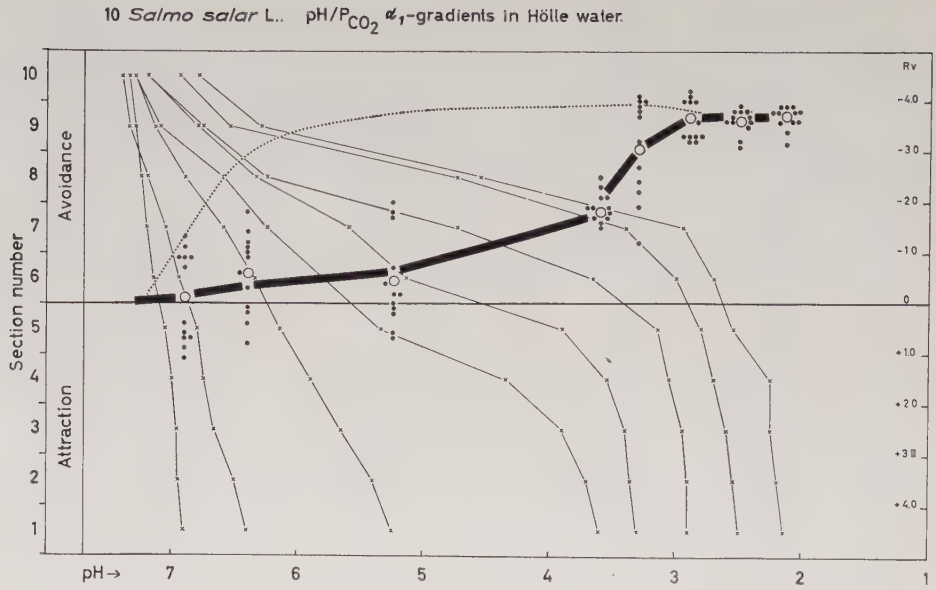


Fig. 30. Reaction diagram (cf. p. 56) showing the reaction curve (heavy one) of salmon parr based on the rv_{5min} (dots) or rv_{60min} (circles) obtained in α_1-gradients of pH/P_{CO_2} of varying steepness in Hölle water. The curve of short dashes is the reaction curve obtained at approximately the same pH gradients established in Uppsala water (cf. Fig. 29). Cf. also p. 96 and Figs. 33, 36, and 37.

the basis of all pH determinations made in the longitudinal sections of the fluviarium, the average pH (small crosses) for sections conveying nearly the same admixtures of base or acid are calculated. Plotted against section numbers (starting with No. 1 at the imaginary top concentration side) these values constitute a series of pH gradients or titration curves, which are drawn in the graph with thin lines. (The corresponding P_{CO_2} values (cf. Fig. 6) are given in Fig. 35 and also in Figs. 31—34). In the framework constructed so far, rv_{5min} (dots) and rv_{1hour} (circles) belonging to each standard gradient are plotted against the lowest or highest pH value, depending on whether HCl or NaOH is supplied. The wide dispersion of the mpv_{5min} from "control periods" (C1, C2, etc. in Fig. 12), shown in Fig. 29, is probably due to after-effects from preceding gradient periods (cf. p. 58). The preference reactions in the actual kind of feed-water, viz. Uppsala water in the case of Fig. 29 and Hölle water in Fig. 30, are visualized by a heavy reaction curve. The reactions in nearly the same pH gradients obtained in the other kind of feed-water, viz. Hölle water in Fig. 29 and Uppsala water in Fig. 30, are shown by a thin reaction curve of short dashes. This graph-drawing method is not quite satisfactory, however, as exactly coinciding titration curves are not obtained in feed-waters with different bicarbonate contents.

Table 13. Reactions of salmon parr (about three months old) in pH gradients in Hölle water.

Experimental conditions		Average pH in section number										Rv_{5min}		
Gradient periods	Time in minutes	1	2	3	4	5	6	7	8	9	10	Range	Mean	No.
A1+K1...	60	6.4	5.6	3.5	3.0	2.6	2.5	2.4	2.3	2.2	2.2	— 2.8	— 4.4	— 3.9	12
A2+K2...	60	5.4	4.2	3.0	2.6	2.4	2.3	2.2	2.1	2.0	2.0	— 3.5	— 4.5	— 4.3	12
A3	30	3.5	3.0	2.5	2.3	2.1	2.0	1.9	1.9	1.8	1.8	— 2.1	— 3.9	— 3.2	6[1]

[1] The experiment was interrupted on account of intoxication. The first signs of balance disturbances were observed about 6 to 7 minutes after the start of A3.

(2) To give the main account of the role played by CO_2, another type of reaction diagrams (Figs. 36 and 37) is constructed with the aid of the auxiliary graphs in Fig. 35. The striated areas in Fig. 35 are limited by two horizontal lines, which represent the lateral walls of the trough, and two vertical lines. The line to the left signifies the CO_2 tension at pH 7.5, which is in equilibrium with the content of dissolved bicarbonate, the right-hand one the maximum obtainable CO_2 tension after acidification, provided no gas escapes from the liquid phase (cf. p. 35). The area limited by the left-hand vertical line, the bottom horizontal one, and the sequence of P_{CO_2} steps, in per cent of the total striated area is called the *degree of CO_2 contamination*. This gives a relative measure of every CO_2 gradient and must be related to the maximum obtainable P_{CO_2}, viz. c. 77 mm Hg in Uppsala water (Fig. 36) and c. 7 mm Hg in Hölle water (Fig. 37). The figures on the parallel, vertical "gradient lines" in Figs. 36 and 37 refer to the actual mean pH in every section. Equal pHs are joined by curves of short dashes, i.e. pH isopleths. The shapes and mutual distances between the pH isopleths are determined by the amounts of acid distributed over the width of the test yard and the buffering capacity of the feed-water. In this type of reaction diagram, rv_{1hour} are plotted against the degree of CO_2 contamination and at the same time against the accompanying pH.

In experiments performed with small salmon parr (about three months old) the results presented in Figs. 30 and 34 were well reproduced. These specimens were also tested in Hölle water in comparatively "pure" pH gradients with rather low pH values (Table 13). The directive influence of CO_2 can be disregarded in the latter case on account of the existence practically all over the test yard of comparatively small amounts of free CO_2 (about 7 mm Hg).

7 *Leuciscus rutilus* (L.). pH/P_{CO_2} α_1-gradients in Uppsala water.

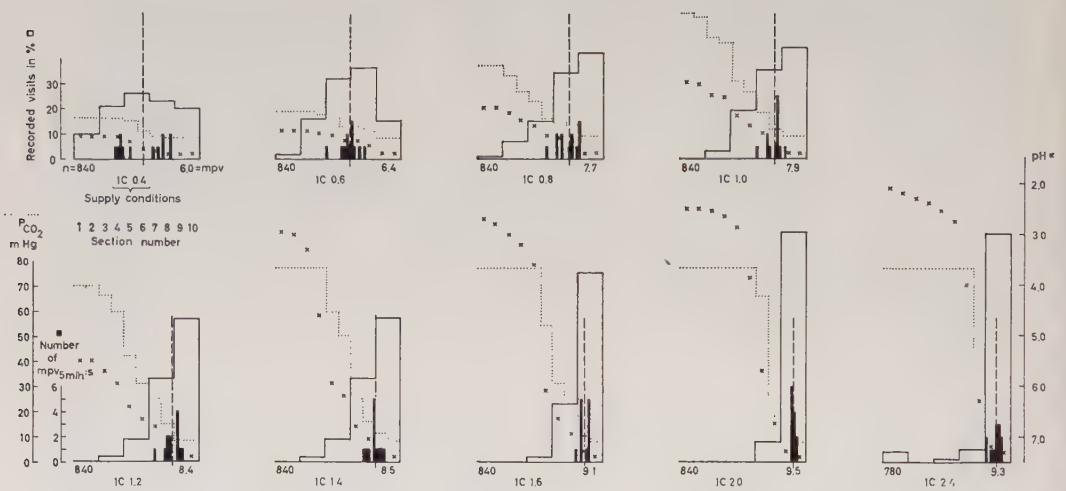


Fig. 31.

Figs. 31—34. Serial graphs of frequency histograms (cf. pp. 46 and 57) showing the recorded visits in five hypothetical double sections of the test yard (Fig. 1). Roach and salmon parr were tested in variously steep α_1-gradients of pH/P_{CO_2} in Uppsala and Hölle water. P_{CO_2} (dotted lines) and pH (crosses) are plotted against section number. Black areas show the distributions of mpv_{5min}. Cf. Figs. 12, 29, 30, 36, and 37.

10 *Leuciscus rutilus* (L.). pH/P_{CO_2} α_1-gradients in Hölle water.

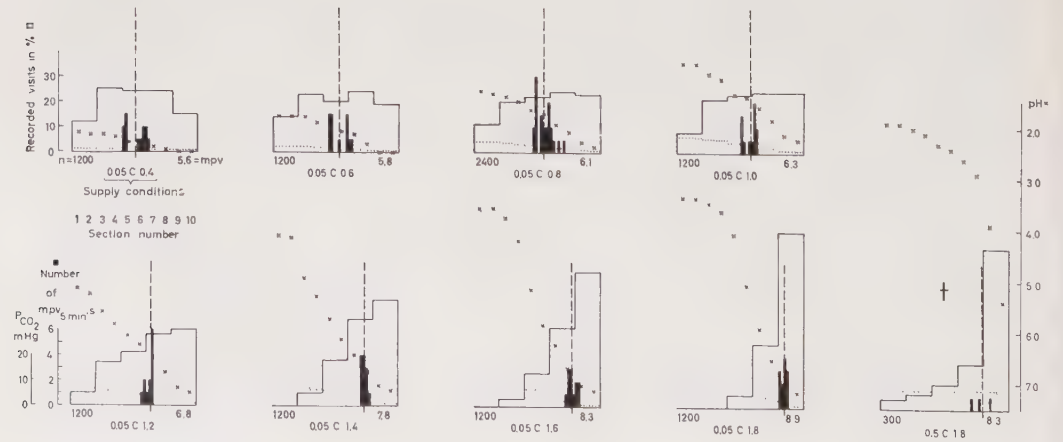


Fig. 32.

10 *Salmo salar* L.. pH/P_{CO_2} α_1-gradients in Uppsala water.

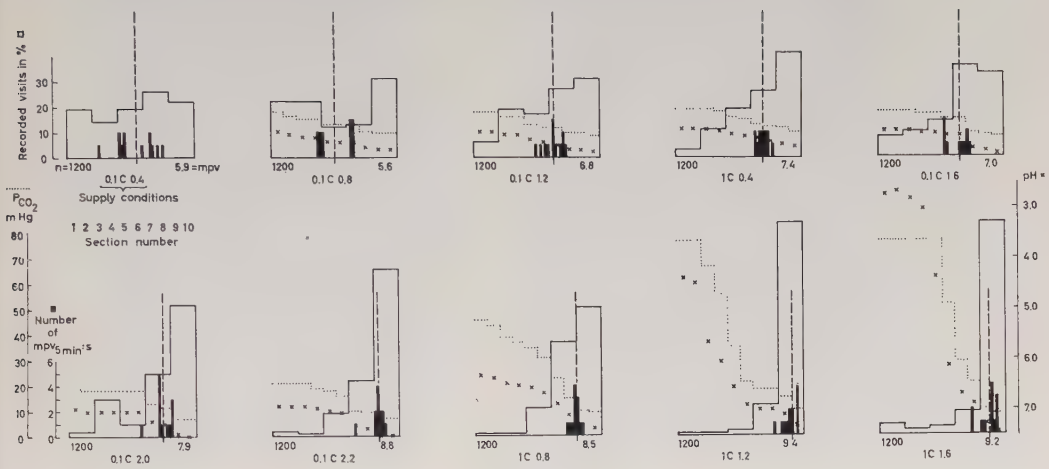


Fig. 33.

10 *Salmo salar* L.. pH/P_{CO_2} α_1-gradients in Hölle water.

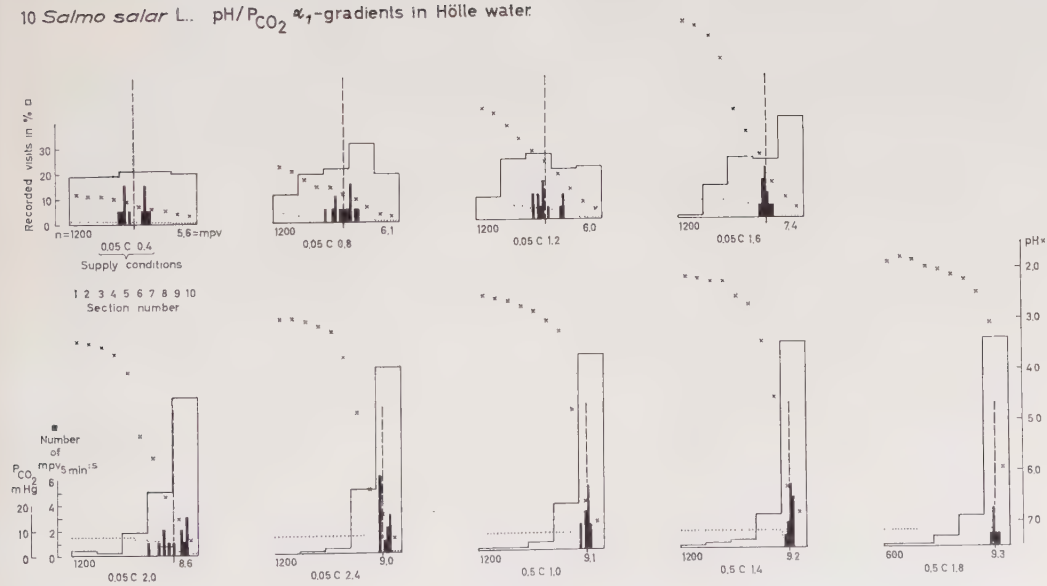


Fig. 34.

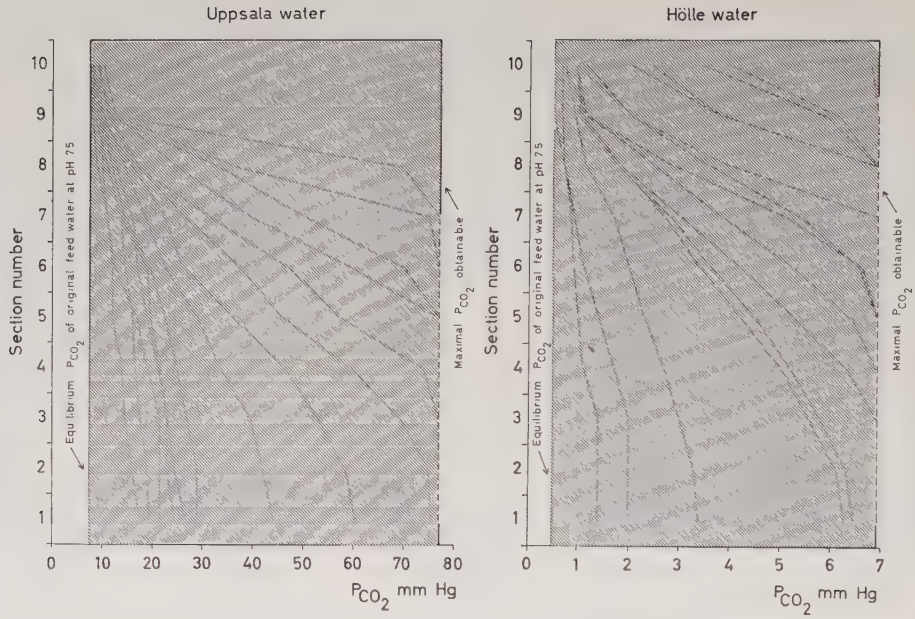


Fig. 35. Auxiliary graph for the construction of the reaction diagrams of Figs. 36 and 37. Considering different standard gradients of α_1-type established in Uppsala and Hölle water, the mean P_{CO_2} from all experiments at the same standard conditions are plotted against the section number of the test yard (Fig. 1). Cf. p. 99.

C. **Discussion and conclusions**

1. *Observations on changed behaviour*

In contrast to the situation described in an abrupt concentration step of oxygen (p. 90) all fish show very marked avoidance reactions in pH/P_{CO_2} gradients, especially in Uppsala water. Instantaneously on confrontation with a definite concentration step a very typical rebound reaction generally occurs. It will be shown below (p. 106) that this reaction is mainly due to a response to CO_2. One gets the impression that the fish are well aware of the direction of the gradient, particularly when swimming against it. This behaviour was called by SHELFORD and ALLEE (1913) a backing-starting reaction. The present observations agree very well with the description given in the paper cited (p. 231):

> The fish moved forward and acted as though it encountered a sheet-rubber wall which it carried forward for a short distance but which in turn through its elasticity, caused the fish to rebound for an equal distance, probably 2 to 10 mm. In other words, the fish suddenly stopped, backed a very short distance and immediately moved forward the same distance again, usually repeating several times. The fishes sometimes turned back after giving this reaction, and sometimes turned back without giving it, and without any other characteristic movement.

Especially during the first phases of a gradient period a number of other reactions occur (Fig. 38). Some of these can be recognized from survival tests made at different CO_2 tension levels by REUSS (1909, 1910) (cf. Table 15). The stimulating action of moderately increased CO_2 upon the respiratory movements and the general activity of fish is well known, as well as the narcotic effects at higher concentrations (REUSS, 1909, 1910; WINTERSTEIN, 1924; FISH, 1943; and others). Intensified opercular movements, coughing, and gasping (BIJTEL, 1947, 1949), are frequently observed. These reflexes are evidently released upon CO_2 stimulation.

In O_2, pH/P_{CO_2} and strong SWL gradients physostomous fish, e.g. cyprinids and salmonids, often exhale gas bubbles from the mouth and opercular openings. At the same time they usually show increased swimming activity and a rising to the surface. The latter phenomenon is known as "surface breathing" (OSBORN and MUNTZ, 1906) or "Notatmung" (WINTERSTEIN, 1908; DIJKSTRA, 1933; OLTHOF, 1941; and others). This may be considered as part of an emergency reaction which is presumably coupled with a "Gasspucksreflex" (DIJKGRAAF, 1950; and others). It was shown by FÄNGE (1953) that the release of the deflatory reflex (gas resorption) of the euphysoclist swim-bladder can be brought about either by oxygen deficiency or by carbon dioxide poisoning. This is considered by FÄNGE to be coupled with the discharge of impulses from the sympathetic nervous system as part of an emergeny reaction (*sensu* CANNON, 1928). Furthermore, according to FÄNGE (1953, p. 97) "distinct similarities exist between the deflatory reflex in euphysoclists and the eel on the one hand and the "Gasspucksreflex" in physostomes on the other hand" (cf. also FÄNGE and MATTISSON, 1956).

2. *Quantitative and qualitative aspects of the preference reactions in combined pH/P_{CO_2} gradients*

It appears from Figs. 29—30 and Figs. 36—37 that the reaction curves obtained in a series of gradually increasing combined pH/P_{CO_2} gradients are characteristic of particular species, provided that the same feed-water is employed (cf. also Figs. 31—34). All curves show gradually increasing avoidance, which is most marked in Uppsala water. With high precision all species seek out the qualities which afford the most favourable conditions. They remain there and turn back from the adverse conditions also in the strongest gradients. Thus, provided that there is a suitable environment, they survive with very few exceptions in all the kinds of combined pH/P_{CO_2} gradients arranged in the present study. Furthermore, it appears from Table 13 that fairly pure pH gradients with pH lower than 6.4, 5.4, and 3.5, respectively, are directive to salmon parr, even though fatal hydrogen concentrations may prevail over large parts of the test yard. In the two first-mentioned gradients they survive for at least one hour without any visible

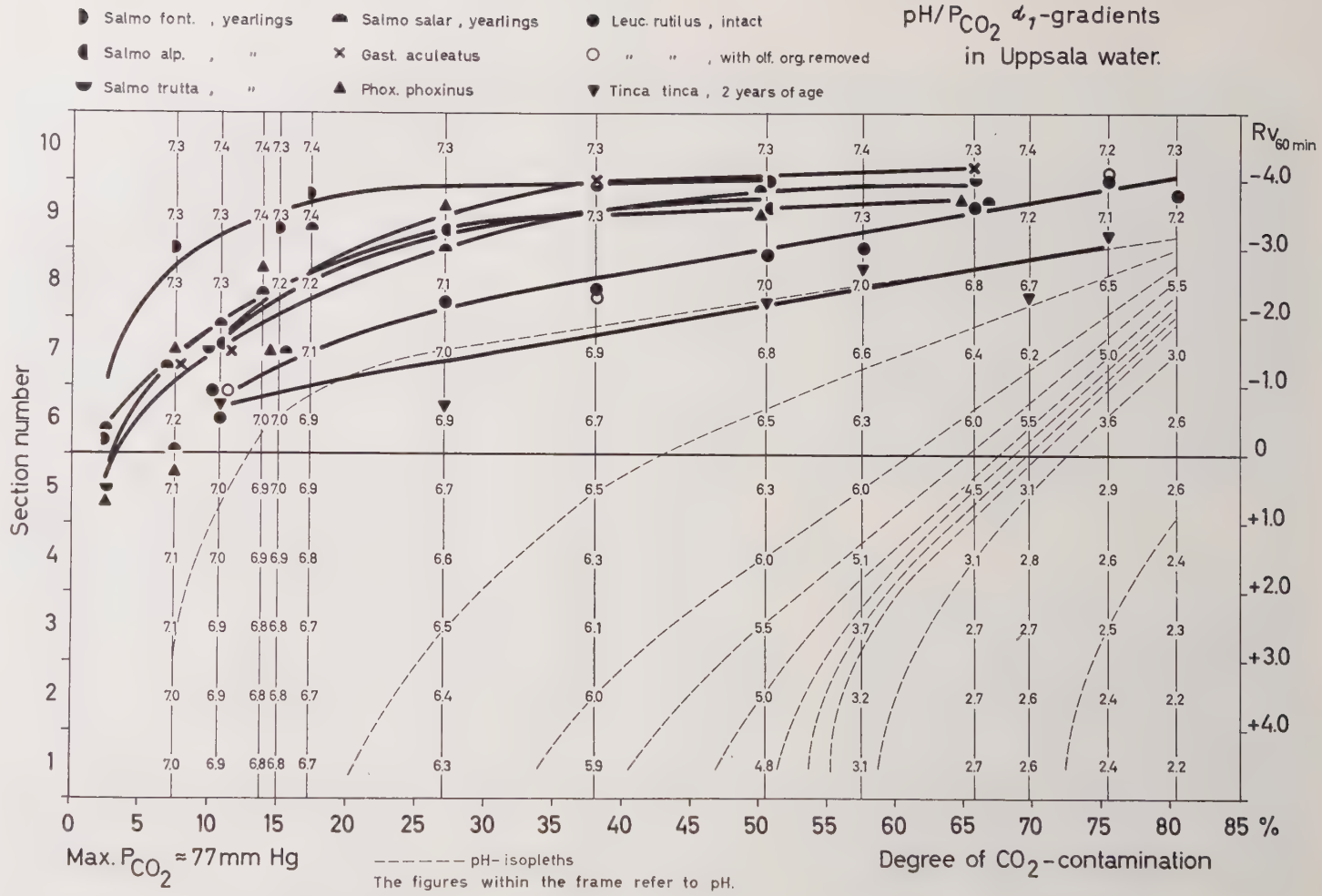

Salmo font. , yearlings
Salmo alp. , "
Salmo trutta , "
Salmo salar , yearlings
Gast. aculeatus
Phox. phoxinus
Leuc. rutilus , intact
" " , with olf. org. removed
Tinca tinca , 2 years of age
pH/P$_{CO_2}$ α_1-gradients in Uppsala water.
Section number
Rv$_{60\,min}$
Max. P$_{CO_2}$ ≈ 77 mm Hg
pH-isopleths
The figures within the frame refer to pH.
Degree of CO$_2$-contamination

Fig. 36. Survey of the reaction curves (heavy ones) of all species studied in α_1-gradients of pH/P_{CO_2} of varying steepness established in Uppsala water. Straight vertical lines connecting figures showing the pH in every section symbolize the different standard gradients employed. Rv_{60min} obtained in various standard gradients are plotted against the degree of CO_2 contamination (for definition, see p. 99) and also visualized against pH (cf. the pH isopleths of short dashes). Cf. further p. 99.

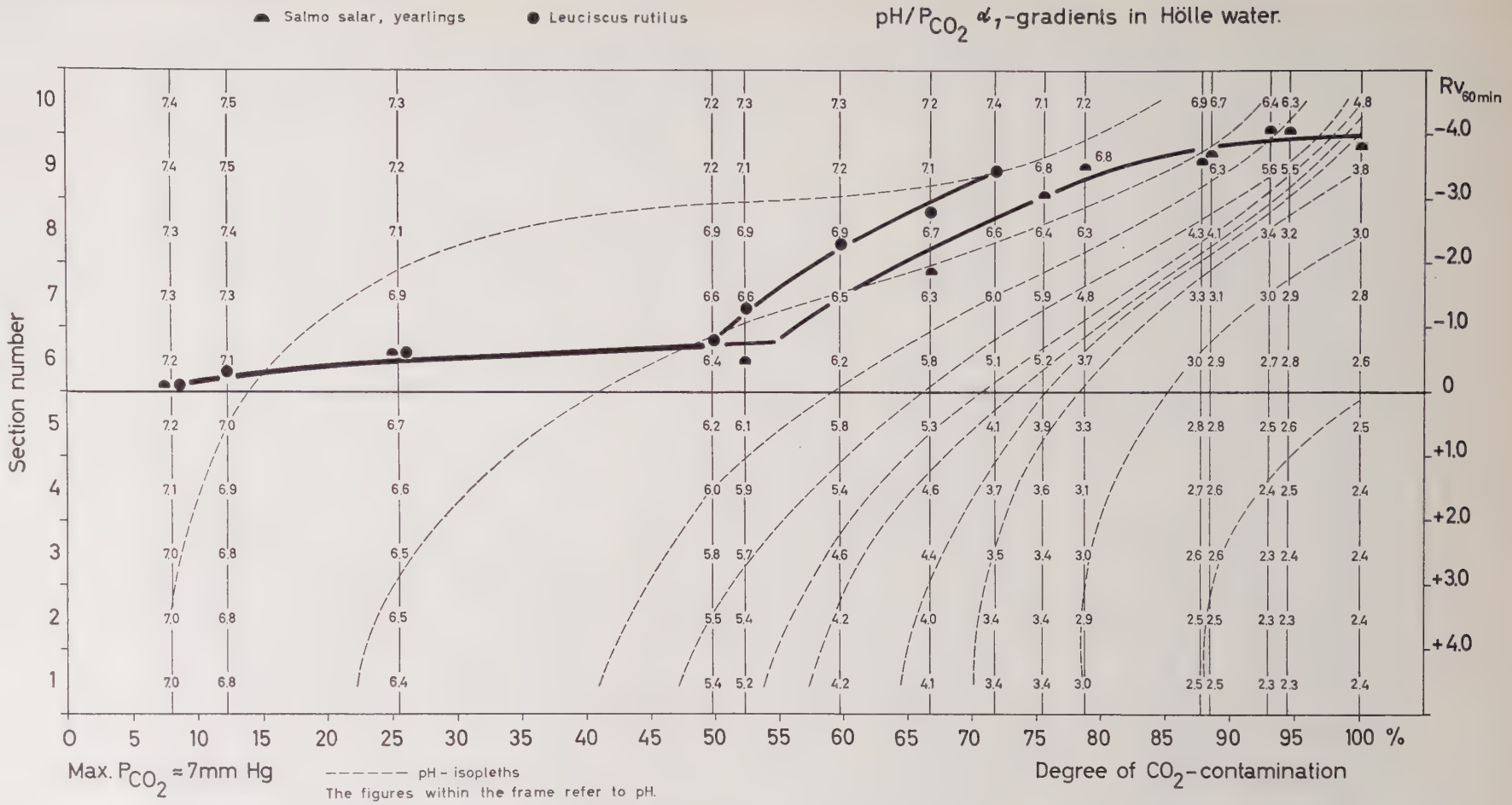


Fig. 37. Reaction curves (heavy ones) of roach and salmon parr studied in steeper and steeper α_1-gradients of pH/P_{CO_2} established in Hölle water. For further particulars cf. Fig. 36 and p. 99.

after-effects, in the third they show avoidance as long as they are able to react altogether, but succumb rather soon.

The course of the reaction curve in Fig. 29 shows that roaches are unable to orientate themselves in hydrogen and bicarbonate ion concentration gradients obtained in Uppsala water within the pH range of about 7.4 to 10.5. The orientative influence of bicarbonate ions can accordingly be disregarded in the present experiments also at lower pH values than 7.4. The formation of some solid compound or compounds gives rise to turbidity at pH higher than c. 10.5. I am not inclined to discuss more definitely the reason for the avoidance stated on the basic side of this pH (Fig. 29). The reaction may or may not be due to high pH and/or some other factor (factors).

Comparing Figs. 29—34, it appears that both roach and salmon give essentially stronger avoidance in Uppsala water than in Hölle water in approximately the same pH gradients. It follows that CO_2 is the main directive factor in combined pH/P_{CO_2} gradients particularly within the pH range of 7.4 to about 5.5. This fact indicates that *fish are able to detect and avoid carbon dioxide separately from the accompanying pH.* It supports earlier suggestions that within a wide range fish are indifferent to pH as a directive stimulus (cf. Powers, 1930, etc.; Jones, 1948; Collins, 1952). According to the results arrived at in the present study (cf. Figs. 29 and 37) this is valid within the pH range of approximately 5.5 to 10.5. This may be compared with the known data about the tolerance limits of freshwater fish to low and high pH, which have been critically reviewed by Doudoroff and Katz (1950). These authors state (p. 1439 and 1436): "It is apparently safe to conclude that in general, the acidity of water with pH values above 5.0 can be tolerated indefinitely by most fresh-water fishes, in the absence of adverse conditions", and ". . . fully developed fresh-water fishes can withstand pH at least as high as 9.0 for long periods. Much higher pH, well above 10.0, also can be tolerated by the more resistant species in the absence of other harmful conditions." Thus, a certain correlation apparently exists between the tolerance limits and the avoidance reactions given to pH.

For a more exact statement of the directive influence of each factor, pH and P_{CO_2}, the reaction diagram of Fig. 37 can be consulted. The reaction curves in this figure are obtained with salmon and roach in Hölle water. They are evidently made up of two elements each. The slightly ascending courses with rising degree of CO_2 contamination found on the left will be attributed to CO_2 (cf. also Figs. 32 and 34). The courses of the two reaction curves coincide, presumably accidentally, in the lower range. This may be due to two counteractive mechanisms, viz. diverse behaviour (cf. Chapter IV) and different CO_2 sensibility [1] (Fig. 36). On the right-hand side of the breaks

[1] The term CO_2 *sensibility* is used here when dealing with the perceptual background to CO_2 orientation. Cf. the foot-note on p. 96.

of the curves the increased steepness can be explained on the assumption that the fishes do not show avoidance reactions to acidity until pHs below a certain threshold value are encountered. This can be determined by the extension to the left of the right-hand curve elements until they meet the horizontal median line of the graph. The vertical projections of the two points of intersection appear in the range of the first section on both sides of the pH isopleth of 5.5. More exactly, we get the incipient levels of pH acting as a directive stimulus at about 5.6 for the roach and about 5.3 for the salmon parr. This means that lower pHs than these values give avoidance reactions.

3. *A comparison of the narcotic*[1] *action of CO_2 and the avoidance in CO_2 gradients among various fish*

The lowest CO_2 tension with narcotic action upon various fish has been determined at about 10°C by REUSS (1909, 1910) and at 19—21°C by WINTERSTEIN (1908). Data presented by these authors are included in Table 14.

Table 14. Carbon dioxide tensions in mm Hg initiating various symptoms among various species of fish.

Species	Excitation	Dyspnoea	Balance disturbance	Initial narcosis	Narcosis	Source
Salmo iridaeus	4 — 8	11 — 15	15 — 30	20 — 35	60	REUSS (1909, 1910)
Salmo trutta	4 — 8	15	15 — 20	20 — 30	60	REUSS (1909)
Perca sp.	—	—	—	—	66.5	WINTERSTEIN (1908)
Leuciscus sp, ...	—	—	—	—	102	Ibid.
Cyprinus carpio ...	8 — 15	20 — 30	85	100	105	REUSS (1909)
Tinca tinca	20 — 30	45 — 50	160	—	180	Ibid.
Carassius sp.	—	—	—	—	234	WINTERSTEIN (1908)

The species are arranged with respect to sensitivity to CO_2. It appears that salmonids are more sensitive than cyprinids. Of the latter the tench is the hardiest species studied in the fluviarium.

As the reactions demonstrated in Fig. 36 are nearly exclusively due to CO_2 (p. 106), a comparison of the order found in Table 14 and the reaction curves obtained in Uppsala water (Fig. 36) give rise to the following consideration. A clear connection exists between the CO_2 tension initiating narcosis among various species and the intensities of their avoidance reactions shown in a series of successively increasing CO_2 gradients.

The following objections, however, can be raised against this method of comparison. (1) Behaviour differences among different species make the reaction curves not quite commensurable (cf. Chapter IV, Fig. 24). The reactions of stickleback, for example, may be exaggerated on account of the sta-

[1] For definition cf. JOHNSON, EYRING and POLISSAR (1954, p. 486).

tionary behaviour and predilection for wall proximity shown by this species. Presumably greater differences exist as regards the acuity of the CO_2 perception between salmonids and stickleback than what is indicated by their respective reaction curves. Judged from the reaction curves, salmonids and stickleback give the sharpest response to CO_2. The minnow follows next with rather undecided reactions, particularly in the weakest gradients. The roach gives essentially lower reactions, and the tench, finally, gives the slightest reactions of all species tested. It is not unjustifiable to say, however, that, if the source of error eminating from different behaviour could be eliminated, the connection between sensibility and sensitivity to CO_2 might have been still more striking. (2) The physiological and toxic action of CO_2 upon fish is complex and far from fully investigated, especially as regards particular species (Fry, 1957, p. 45). One source of error is seasonal changes in the sensitivity to CO_2 (Hart, 1957). This is presumably of slight importance as regards fishes acclimatized for long periods to a definite constant water quality.

In spite of the imperfections in this analysis, it cannot be denied that a real correlation seems to exist between toxic action and directive influence of CO_2 upon the reactions of various fish.

The avoidance in CO_2 gradients may be the result of both an emergency reaction (particularly in the initial phases of a gradient period) and (probably to the greatest extent) of directive stimulation of external CO_2 sensible receptors (Fig. 38). The latter may be coupled to the gustatory sense or to a special CO_2 sense which may be looked for in the gill region (cf. below). The mechanism described has a protective function in the present experiments. This must be advantageous to the species also in extreme biotopes in nature which may have been more frequent in past ages. It has been proposed (Westoll, 1944) that the evolution of teleost fish may primarily have taken place in environments somewhat similar to those existing in the stagnant swamps in the Paraguayan Chaco (Carter and Beadle, 1930, 1931). These waters are characterized by very low oxygen content, but a very large content of CO_2.

4. *The localization of the presumed sensory basis for the avoidance reactions to acidity and carbon dioxide*

The avoidance of acidity is probably due to the stimulation of taste buds (or the common chemical sense) located in the skin and mouth cavity (Herrick, 1903, 1906; Parker, 1912, 1922; Strieck, 1924; Klenk, 1930; Scharrer, 1935; Scharrer, Smith, and Palay, 1947; Hasler, 1957).

On the basis of some experiments with the gradient tank Powers and Clark (1943) arrived at the opinion that the responses of fish to a CO_2 gradient were located in the lateral organs. Similar experiments were made in

Scheme of the essential reactions and mechanisms released in parr stages of Salmo salar in combined pH/P_{CO_2} gradients (partly hypothetical).

	Approximate duration in min.	OBSERVED BEHAVIOUR AND SYMPTOMS increasing ← contamination → decreasing	DISCERNIBLE PHASES increasing ← contamination → decreasing	ETHOLOGICAL ASPECT increasing ← contamination → decreasing	PHYSIOLOGICAL ASPECT increasing ← contamination → decreasing
Arrival of the gradient		Retain bottom positions facing the current	FINAL PHASE with normal behaviour	Modified territoriality	Normal functions
	0–1	D:o. Movements of mouth and opercula Accentuated ventilation	PERCEPTION PHASE	Motivation[1] increases upon external stimulation	CO_2-perception by means of paired(?) chemoreceptors at essentially lower concentrations than those interfering with physiological processes necessary for life (respiration) Warning and protective function
	0–2	Oscillatory and irregular body-movements. The fishes still restricted to stationary bottom positions Ascension starting in the most contaminated water	ACTIVATION PHASE	Further intensification of motivation for free-swimming behaviour Appetitive behaviour	Dyspnoea. Ventilatory movements regulated by reflexogenic areas in the branchial region (Cf. the chemoreceptors of Sin. carot. and Sin. aort. in higher vertebrates) and/or over the respiratory centre
	1–3	Swim at random visiting all parts of the test yard, most lively in higher concentration	EXCITATION PHASE	(Trial and error movements) Restiveness Tranquillity Ortho-kinesis	Emergency reaction: Respiratory distress "Gasspucks-reflexe"
	1–5	Hesitate Stop and turn Movements of lips Rebound, fly back Coughing reflexes } When swimming against the gradient	PREFERENCE PHASE	Directed avoidance, when swimming against the gradient Decreasing motivation for swimming	Sympathetic reaction → Contraction of swim bladder Increased swimming activity Hydrostatic regulation
		Behave very excitedly Exhale gas bubbles through the mouth and gill openings Inhale, when visiting the surface layers? Occupy gradually new bottom positions Decreasing ventilatory movements	PANIC PHASE → STABILIZATION PHASE	Flight reactions Relaxation through lack of stimulation Consummatory act Agonistic behaviour	"Notatmung" at the surface Respiratory paralysis
		Spastic movements Swim jerkily and drop irregularly to bottom or are swept downstream Loss of equilibrium	COMATOSE PHASE → FINAL PHASE with normal behaviour	Normal ethological pattern not functioning Modified territoriality	CO_2 narcosis Asphyxiation Lethal respiratory acidosis Rapidly regained normal respiration
The duration of phases is dependent on the steepness of the gradient			MORS		

Fig. 38. Schematical survey of the essential reactions shown by salmon parr at the first confrontation with the arriving gradient and during the initial period after the establishment in Uppsala water of α_1-gradients of pH/P_{CO_2}.

the fluviarium with salmon parr, roach, and minnow. The lateral nerves were clipped on each side just behind the opercula by two incisions about 5 mm apart. No essential decrease in the avoidance reactions of operated specimens in comparison with intact ones was obtained (Figs. 15 and 36). The statement by POWERS and CLARK that CO_2 receptors may be located in the lateral line organs is obviously unfounded.

It is also shown (Fig. 36) that the removal of olfactory tissue does not affect the ability of roaches to avoid CO_2. Unfortunately, on account of the difficulties of eliminating the taste organs and the common chemical sense, as well as extirpating the sensory nerves from the region of the gill arteries, no similar tests can conveniently be made regarding these senses.

Thus the lateral line and the olfactory organs cannot be responsible for the ability to detect CO_2.

Comparing the results arrived at in Uppsala water (Fig. 36) and those in Hölle water (Fig. 37) it appears that the reaction curves of roach and salmon have changed positions as regards the average distance to the median zero line. The sharpest avoidance reactions to CO_2 are given by the salmon (Fig. 36) and to pH by the roach (Fig. 37). This fact cannot be connected with changed behaviour. It indicates that acidity and CO_2 are perceived either (1) by the same receptors at essentially different thresholds by the two species or (2) by different receptor systems.

(1) In the first alternative both acidity and CO_2 are presumably perceived by the gustatory sense.

(2) If the second alternative holds good, there would be reason to presume the existence of a special CO_2 sense. The respiratory movements are considered to be partly controlled by reflexes released upon the stimulation of CO_2-sensible receptors in the gill epithelium (cf. DIJKSTRA, 1933; POWERS and CLARK, 1943; BLACK, 1951; and others). The increased ventilation and frequency of the cleaning reflexes observed in the present experiments before a real preference is obtained (p. 103 and Fig. 38) possibly support the assumption that the environmental CO_2 is perceived by external receptors. It seems therefore plausible, in this connection, to pay fresh attention to the possibility that the presumed CO_2 sense may be connected with CO_2-sensible chemoreceptors located in the gill region. These may be homologous with those found in the carotid sinus and aortic body among higher vertebrates. Suggestions along this line have repeatedly been advanced in the discussion emanating from the fundamental results published in the early thirties by HEYMANS and coworkers. However, no conclusive evidence for the presence in the gill arteries of fish of a mechanism similar to that of the reflexogenic areas in the vascular system of mammals seems yet to have been published (cf. BEHRENS and HIKIJI, 1933; BOYD, 1936; POWERS and CLARK, 1942, 1943; SCHMIDT, 1941; MOTT, 1951, 1957; HEYMANS and NEILS, 1958, p. 186 ff.; and others).

The hypotheses expressed in the second alternative discussed here are still open to discussion. More light might possibly be shed on the problem by future fluviarium experiments. Then the gustatory sense or the presumed CO_2-sensible chemoreceptors ought to be eliminated by using some kind of a locally and specifically acting anaesthetic or by making the necessary, but rather complicated nerve eliminations.

III. Reaction to sulphite waste liquor (SWL)

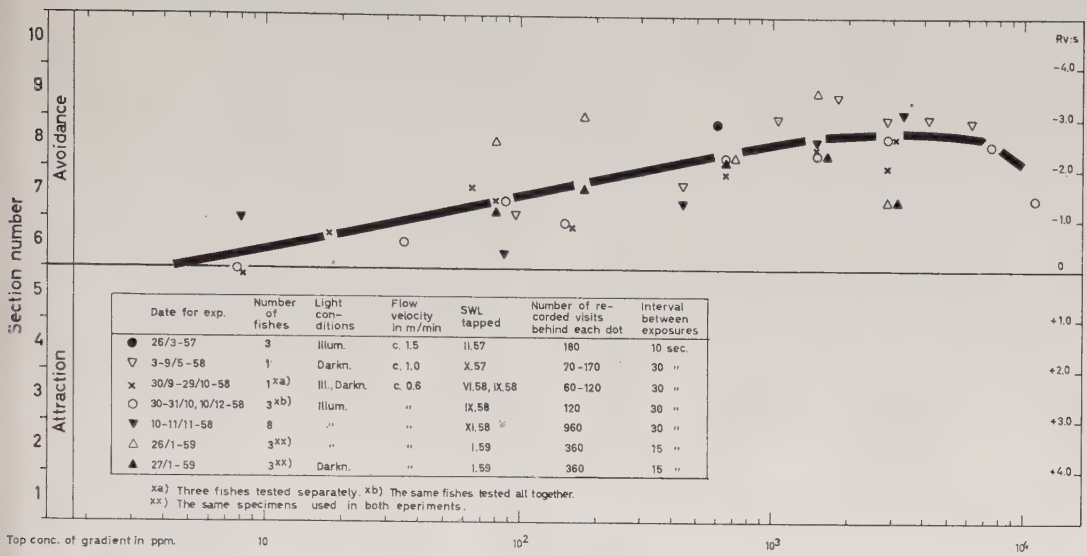
A. Background

As far as the present author is aware, the avoidance responses to SWL have previously been studied only twice. JONES, WARREN, BOND, and DOUDOROFF (1956) used an avoidance tank partioned at one end into four open parallel channels (cf. p. 12). Two of these contained unadulterated water and the other two water mixed with SWL. Parr stages of chinook salmon (*Oncorhynchus tshawytscha*) showed marked avoidance at the confrontation with the abrupt concentration step of 2000 ppm and progressively decreasing reactions down to 250 ppm. A few tests with young coho salmon (*Oncorhynchus kisutch*) indicated little or no avoidance to SWL at 1000 and 2000 ppm. Using an apparatus similar to that constructed by COLLINS (1952), SMITH and SAALFELD (1955) found that river smelt (*Thaleichtys pacificus*) avoid a mixed sulphite and sulphate effluent. The reaction threshold is said to lie at a dilution of 1 : 800. This statement is of little value, however, on account of undefined experimental conditions.

B. Experiments and results

A comparative study has been performed on the basis of the reactions shown by a number of fresh-water fish in similarly arranged experimental series of steeper and steeper α_1-gradients of SWL obtained in Uppsala water (Figs. 39—43 and 45—51) and in Hölle water (Fig. 44). The chemical and technicological definitions of the liquors employed are given in Chapter II (Table 7). Other experimental data are to be found in Table 1 and in connection with the relevant figures presenting the results (Figs. 39—51). The following species were tested, viz. *Phoxinus phoxinus* (Figs. 39—41), *Leuciscus rutilus* (Figs. 42—46), *L. idbarus* (Fig. 47), *Perca fluviatilis* (Fig. 48), *Coregonus nasus* (Fig. 49), parr stages of *Salmo salar* and other salmonids (Fig. 50), and *Gasterosteus aculeatus* (Fig. 51). All quantitative information arrived at in the experiments has been entered on reaction diagrams constructed as described on p. 56. As complements to the reaction diagrams of Fig. 42 and 43 the results arrived at with intact roaches and roaches with

SWL. *Phoxinus phoxinus* (L.) tested under different experimental conditions. Uppsala water. α_1.



	Date for exp.	Number of fishes	Light conditions	Flow velocity in m/min	SWL tapped	Number of recorded visits behind each dot	Interval between exposures
●	26/3-57	3	Illum.	c. 1.5	II.57	180	10 sec.
▽	3-9/5-58	1	Darkn.	c. 1.0	X.57	70-170	30 "
×	30/9-29/10-58	1 xa)	III., Darkn.	c. 0.6	VI.58, IX.58	60-120	30 "
○	30-31/10, 10/12-58	3 xb)	Illum.	"	IX.58	120	30 "
▼	10-11/11-58	8	"	"	XI.58	960	30 "
△	26/1-59	3 xx)	"	"	I.59	360	15 "
▲	27/1-59	3 xx)	Darkn.	"	I.59	360	15 "

xa) Three fishes tested separately. xb) The same fishes tested all together.
xx) The same specimens used in both experiments.

Fig. 39.

Fig. 39—41. Reaction diagrams (cf. p. 56) showing rv plotted against the logarithm of the top concentration of α_1-gradients of SWL esfablished in Uppsala water. To the left in Figs. 40—41 mpv_{5min} in control conditions are plotted against the section number. Dilution of SWL and aperture diameter of the outlet from Marriotte's flask (Figs. 1 and 5) are given as pairs of figures after "Exp. conditions". For the chemical definition of SWL see Table 7.

eliminated olfactory organs are also presented in the form of serial diagrams of frequency histograms (Figs. 45 and 46). Figs. 11 and 18, finally, give a survey of some experiments with roach and perch.

C. **Discussion and conclusions**

1. *Minnow*

Quite well-reproducible reaction curves were obtained in seven series of experiments with *Phoxinus phoxinus,* although other external conditions than the environmental gradients varied from series to series. This is shown in Fig. 39. In more standardized experimental conditions a still smaller variation might be expected.

In order to study the differences in excitability among individuals a group of three minnows was used in two parallel series (Figs. 40 and 41). The results presented in Fig. 40 were obtained when the fishes were tested one by one. Almost exactly the same reaction curve was obtained when the three

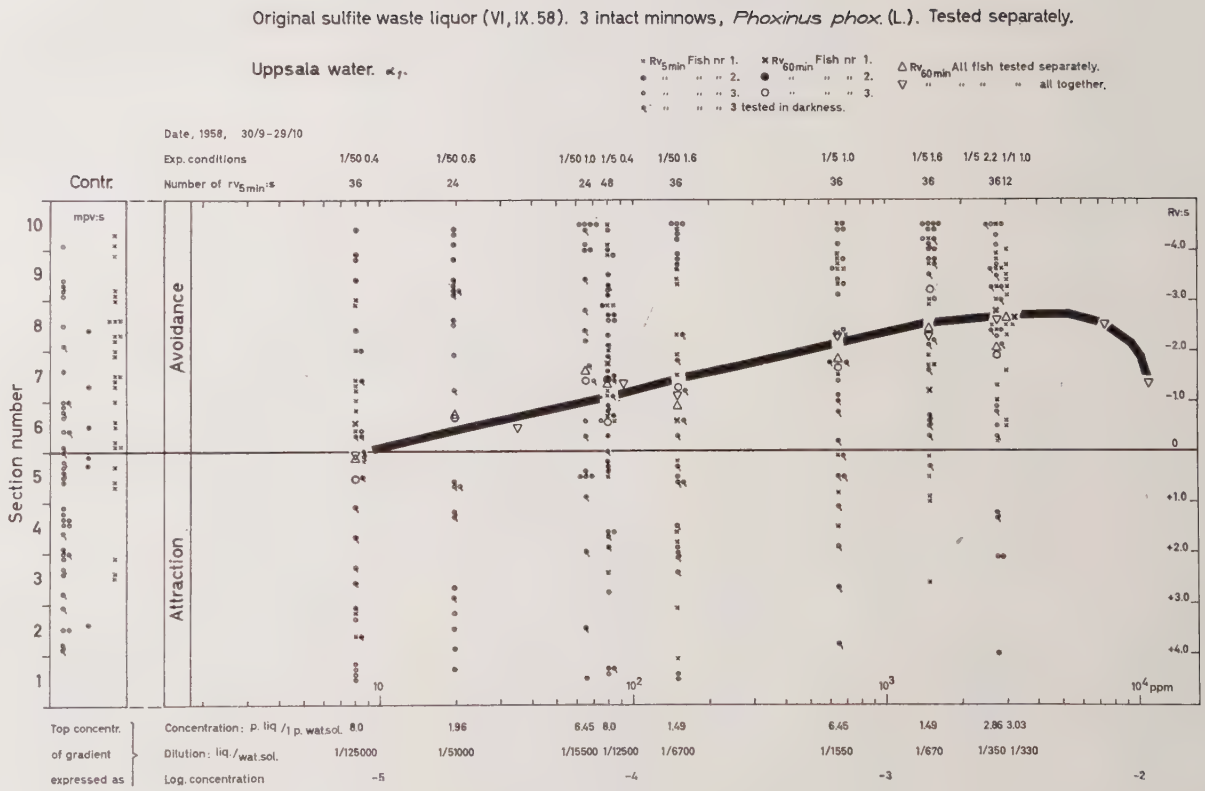


Fig. 40. Cf. Fig. 39.

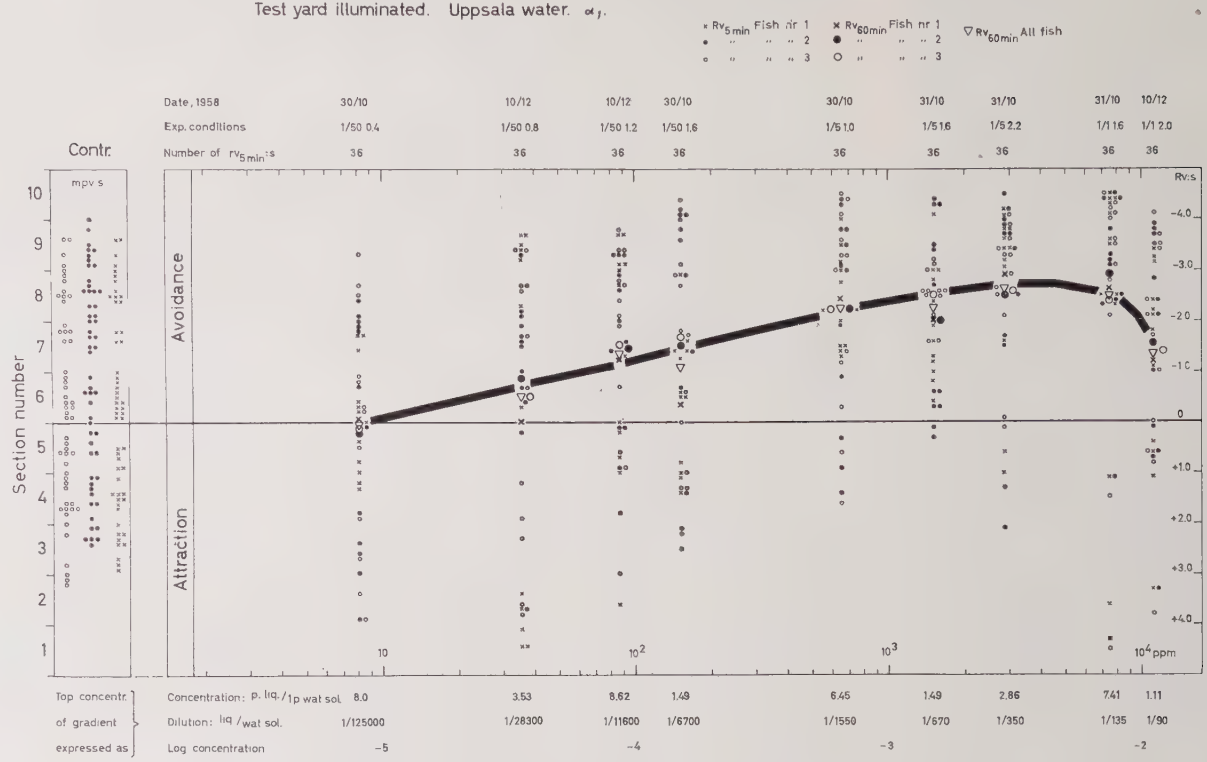


Fig. 41. Cf. Fig. 39.

specimens were tested all together in the same types of gradients (Fig. 41). The dispersion among mpv_{5min} for particular specimens and standard gradients was greater when the fish were tested separately than when they were tested all together. This evidently depends on the pronounced schooling behaviour which is particularly typical of this species (cf. p. 86). No clear tendency of any particular fish to show reactions deviating from the average ones is found.

It is reasonable to say that the shape of the reaction curve drawn in Figs. 39—41 is quite characteristic of the minnow under the actual experimental conditions. The course of the curve as regards the stimulating influences upon the reactions from the environmental gradients, will be discussed in more detail in connection with the explanation given of the course of the reaction curve arrived at in the experiments performed with roaches (p. 115).

The nature of the avoidance underlying the reaction curve of minnow is furthermore elucidated by the results shown in Table 15. The same three minnows were used in three similarly arranged experimental series run in Uppsala water from Jan. 26 to Feb. 2, 1959. At that time the test animals had been held in the storing basins within the laboratory for more than two years. In the first two series the specimens were intact and tested in gradients of original SWL (second column in Table 15) and of neutralized SWL (third column). After the occlusion of their olfactory organs they were again tested in gradients of original SWL (last column). By the hypothesis that avoidance to SWL is mainly due to olfactory stimulation, rv near nil might be expected in all cases dealt with in the third column of Table 15. The rather inconsistent reaction values obtained in these series may be due to decreased vitality caused by the surgical incisions and the previous use of the fish in the preceding series of experiments. Loss of test fish at the time prevented a more complete experimental treatment of the problems.

Table 15. Rv_{1hour} attained during two 30-minute periods with corresponding inversed α_1-gradients of SWL (I 59; cf. Table 7) at each concentration level. Three *Phoxinus phoxinus* were tested at a time. The same specimens were used in all experiments.

Top concentration of gradient in ppm	Intact fishes		The same specimens with olfactory organs eliminated
	Original SWL	SWL neutralized to pH 7.4	Original SWL
80	— 1.8	— 0.6	— 0.1
180	— 2.4	— 1.7	+ 0.5
650	— 2.2	— 1.4	+ 0.4
1500	— 2.9	— 1.5	— 0.3
2900	— 1.3	— 1.3	+ 0.2

Anyhow, the data presented in Table 15 give rise to the following conclusions and suggestions. (1) The avoidance of SWL is connected with olfactory sensations. (2) Any CO_2 effects (cf. pp. 102—107 and Fig. 36) are not visible in the tests with operated fish. (3) The avoidance reactions decrease, but are still pronounced when neutralized SWL is used in the experiments instead of original SWL. The difference of reaction may indicate that the avoidance is partly due to the $SO_2/HSO_3^-/SO_4^{2-}$ protolytic system or presumably mainly to SO_2. Further experiments are needed to verify this assumption. (4) The remaining reactions shown in gradients of neutralized SWL are possibly not due to this system, but rather to some aromatic compound (compounds) or other odorous components of SWL.

2. *Roach*

The preference reactions of intact roaches obtained in SWL α_1-gradients of varying steepness established in Uppsala water are shown by the reaction curve in Fig. 42. The course of this curve was in all essentials well reproduced in another series with somewhat bigger intact roaches (cf. Table 1).

The olfactory epithelia of five specimens considered in Fig. 42 were burned out with a red-hot nail (Marcström, 1959). After the removal of the olfactory organs the main part of the avoidance shown in Figs. 42 and 45 failed to appear. This is demonstrated in Figs. 43 and 46.

This result is in agreement with the observations made on minnows (cf. Table 15). This evidence may give rise to a more general conclusion, viz. that the avoidance of fish in SWL gradients is mainly released upon olfactory stimulation deriving from some odorous component (components) in the liquor. This suggestion will be further elucidated in the following discussion.

It appears from Fig. 43 that the remaining avoidance reactions to SWL shown by roaches after the removal of their olfactory organs are exclusively found in the steepest gradients, that is, when comparatively large amounts of SWL are added to the fluviarium. In these cases interaction between free hydrogen ions added to the liquor and the bicarbonate ions of the feed-water gives rise to considerable pH/P_{CO_2} gradients. This is shown in the lowest horizontal scale of some reaction diagrams stating the pH values measured *in situ* on the top concentration side of the test yard. It was shown earlier (p. 109 and Fig. 36) that the elimination of the olfactory sense does not impair the ability of roaches to avoid the higher concentrations of pure pH/P_{CO_2} gradients. From Figs. 29, 31, and 36, it appears that, in the case of Uppsala water and intact roaches, a combined pH/P_{CO_2} gradient with a pH on the top concentration side of about 7.0 will give rise to a negative reaction value of one-half to one unit (section breadth = one-tenth of the width of the test yard). This is in good agreement with the intensity of avoidance reaction arrived at in the steepest SWL gradient (with a minimum pH of 7.0)

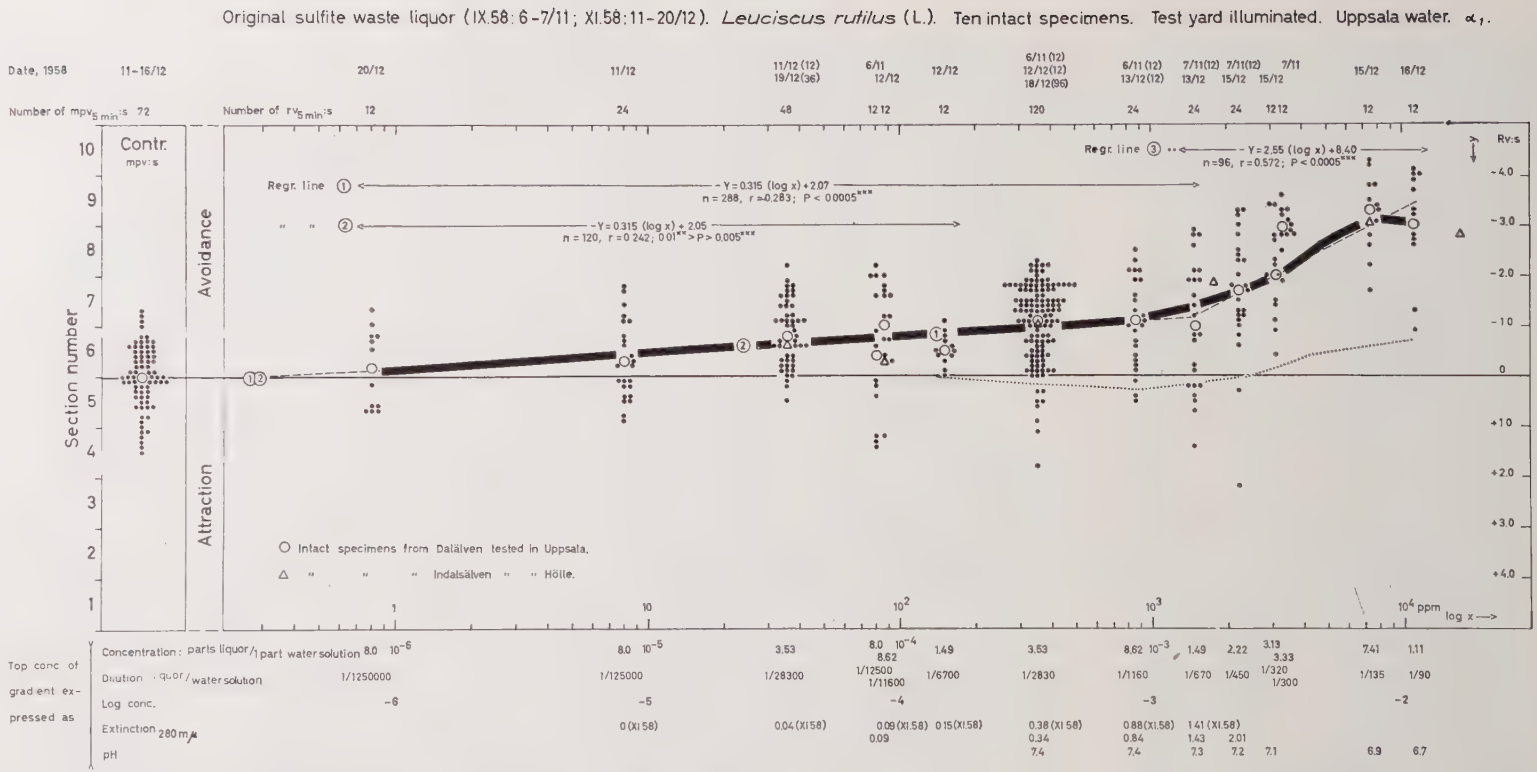

Original sulfite waste liquor (IX.58: 6-7/11; XI.58: 11-20/12). Leuciscus rutilus (L.). Ten intact specimens. Test yard illuminated. Uppsala water. α1.
Date, 1958
Number of mpv5 min:s 72
Number of rv5 min:s
Contr. mpv:s
Avoidance
Attraction
Section number
Regr. line ① Y = 0.315 (log x) + 2.07 n = 288, r = 0.283; P < 0.0005***
Regr. line ② Y = 0.315 (log x) + 2.05 n = 120, r = 0.242; 0.01** > P > 0.005***
Regr. line ③ Y = 2.55 (log x) + 8.40 n = 96, r = 0.572; P < 0.0005***
Rv:s
O Intact specimens from Dalälven tested in Uppsala.
Δ " " " Indalsälven " " Hölle.
ppm
log x
Top conc of gradient expressed as
Concentration: parts liquor/1 part water solution
Dilution: liquor/water solution
Log conc.
Extinction 280 mμ
pH

Fig. 42. Reaction diagram (cf. p. 56) showing rv plotted against the logarithm of the top concentration of α_1-gradients of SWL established in Uppsala water. To the left mpv in control conditions are plotted against section number. Dots denote rv_{5min} and mpv_{5min}. Circles and triangles (cf. Fig. 44) denote rv and mpv based on all records made in the same standard gradient. The actual reaction curve (heavy one) is drawn along with the reaction curve of Fig. 43 (dotted one). Regression lines are calculated on all rv_{5min} within three different concentration ranges as indicated by thin lines and arrows with pertinent equations and other statistics. The centre coordinates are shown by encircled ordinal numbers. The almost coinciding regression lines of No. 1 and No. 2 are extrapolated to the left. The intersection with the median line of the graph is marked out by a double circle (1, 2), which may give an estimate of the actual reaction threshold. Cf. also Fig 45.

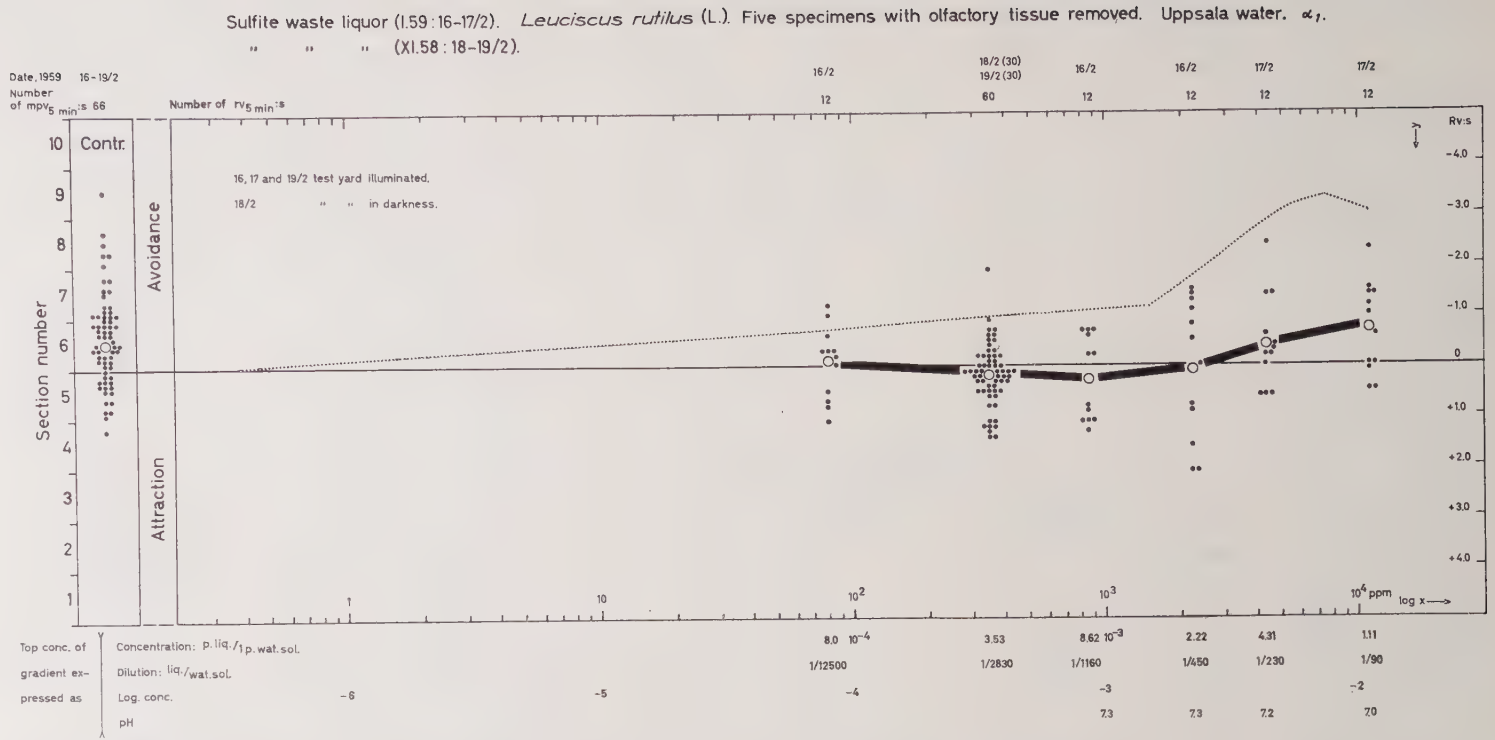


Fig. 43. Reaction diagram (cf. p. 56 and Fig. 42) of roaches with eliminated olfactory organs. The dotted curve is the reaction curve of Fig. 42. Cf. also Fig. 46.

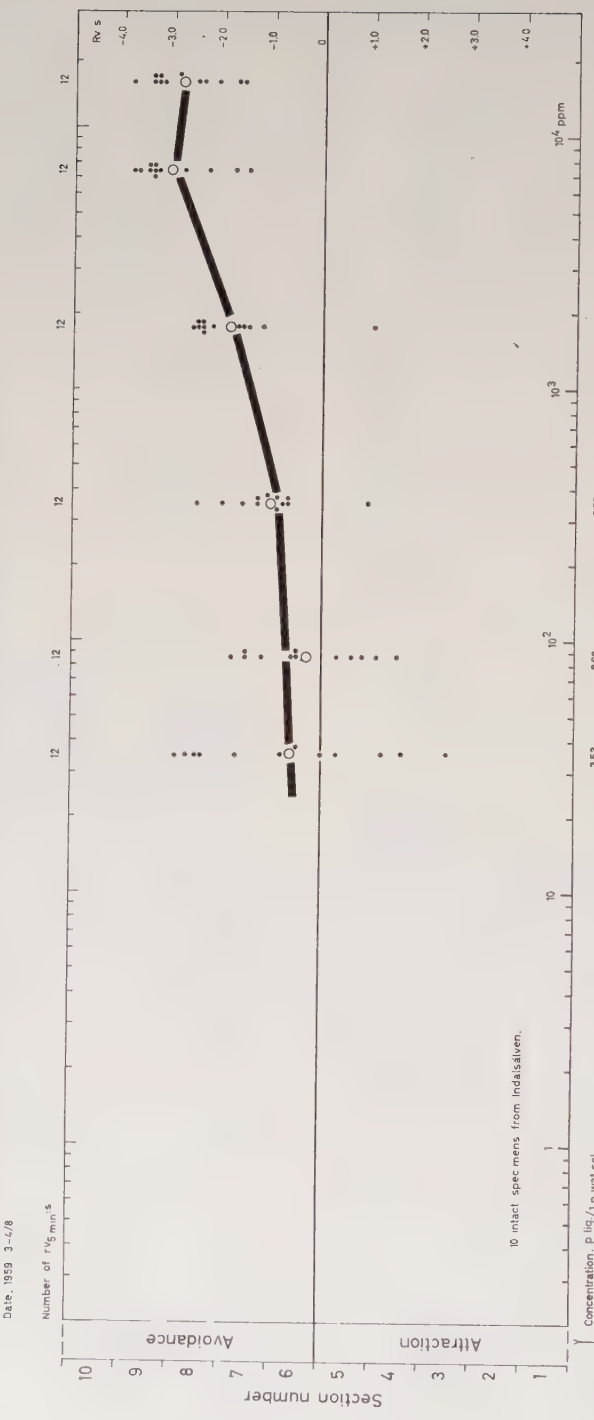


Fig. 44. Reaction diagram (cf. p. 56 and Fig. 42) showing the preferences of intact roaches in α_1-gradients of SWL which were established in Hölle water.

considered in Fig. 43. Accordingly, the avoidance of SWL gradients by roaches with their olfactory organs removed is very probably due merely to pH/P_{CO_2} gradients. Along this line of interpretation the part of the avoidance shown by intact roaches in SWL gradients which is not due to pH and CO_2 can be entirely attributed to reactions released upon olfactory stimulation. The slight attraction visible in the medium of the heavy reaction curve of Fig. 43 can hardly be significant.

The directive influence of pH and CO_2 upon the avoidance reactions in SWL gradients was furthermore studied by the performance of a series of experiments with intact roaches in Hölle water. The results are entered on the reaction diagram of Fig. 44. It appears in a comparison between Figs. 42 and 44 that reaction curves of fairly coinciding shapes are obtained in steeper and steeper SWL gradients established in Uppsala and Hölle water. This result might at first sight seem to be rather confusing, as the addition of the same amount of SWL to the two kinds of feed-water gives rise to very different pH/P_{CO_2} gradients (cf. Fig. 6). But considering the problem more closely it may be explained upon the assumption that the actual reactions are due to both olfaction and response to CO_2 and/or pH. Up to a top concentration of about 1000 ppm SWL the gradually ascending negative reactions, independently of water quality, are evidently merely due to olfaction (cf. Fig. 43 and Walker and Hasler; 1949; Hasler and Wisby, 1951). At the top concentration mentioned (about 1000 ppm) a break in the reaction curve of Fig. 42 is visible. The steeper-rising avoidance reactions with increasing sleepness of gradient, which is indicated from 1000 ppm up to a top concentration of about 7500 ppm, may be explained upon the assumption that the directive influences from odorous substances, pH and CO_2 combine. It cannot be denied, however, that the typical mobility of the fish in combination with a preference for a certain part of the confined space (cf. p. 69 and Fig. 45) might equally well induce such a bend in the reaction curve. The similarity of the two curves (Figs. 42, 44) is explained in the following way. In the Uppsala water the effect of CO_2 is predominant in the steepest SWL gradients (cf. p. 106 and Fig. 36). In the more poorly bicarbonate-buffered Hölle water, on the other hand, the situation is more complicated. Below pH about 5.6, pH is probably the main directive factor (cf. p. 107 and Fig. 37). At higher pH CO_2 effects may occur as well, besides the effects of odorous stimulations.

It seems reasonable to presume that somewhere within the top concentration range between 1000 and 7500 ppm, the reactions due to olfaction culminate. This is indicated by the descent at the extreme righthand ends of the reaction curves presented in Figs. 39—42, and 44. If the influence of CO_2 upon the course of the reaction curves in these figures could be disregarded, the culmination of the olfactory effects would be still more striking. The actual regress of olfactory response may depend upon more or less pro-

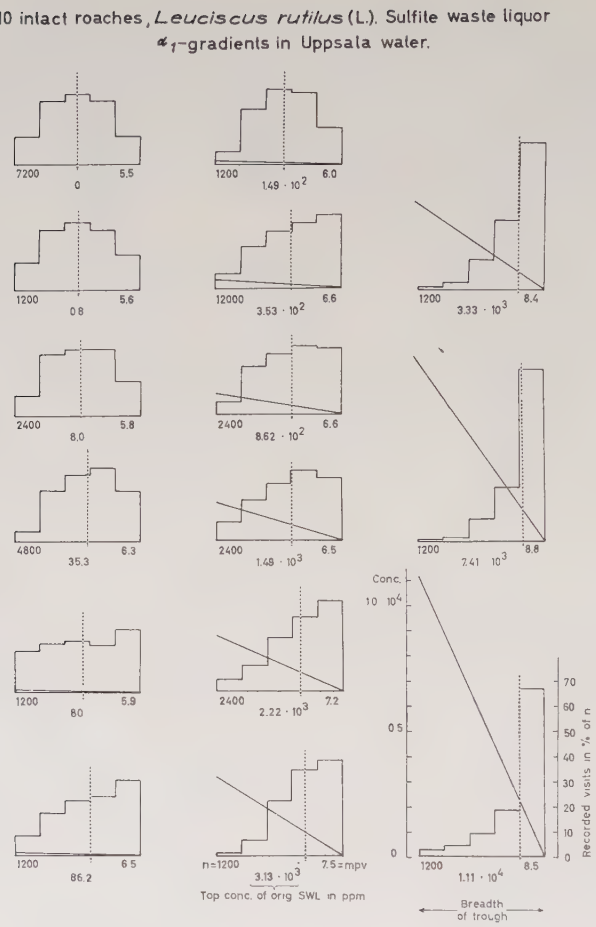


Fig. 45.

Figs. 45—46. Serial graphs of frequency histograms (cf. pp. 46 and 57) showing the recorded visits of intact roaches (Fig. 45) and roaches with their olfactory organs removed (Fig. 46) in five hypothetical double sections of the test yard in standard gradients of SWL of varying steepness (cf. Figs. 42 and 43). The gradients of α_1-type (cf. Fig. 1 and Table 2) are schematically represented by straight lines of increasing inclination. The top concentration (expressed in ppm as stated by figures at the bottom of the histograms) is always found to the left. The mpv based on all observations (n) in every standard condition are given by dotted vertical lines and figures on the right at the bottoms of the histograms.

nounced exhaustion of the receptors. Dealing with the reactions shown by roaches in weaker α_1-gradients of SWL (at the top concentration of about 350 ppm) it was pointed out (cf. p. 58 and Fig. 11), however, that fairly constant rv_{5min} were obtained during the course of the experiments, though the test fishes stayed in the gradient for several hours. But a gradually diminishing capacity of the fish to discriminate between the olfactory sensa-

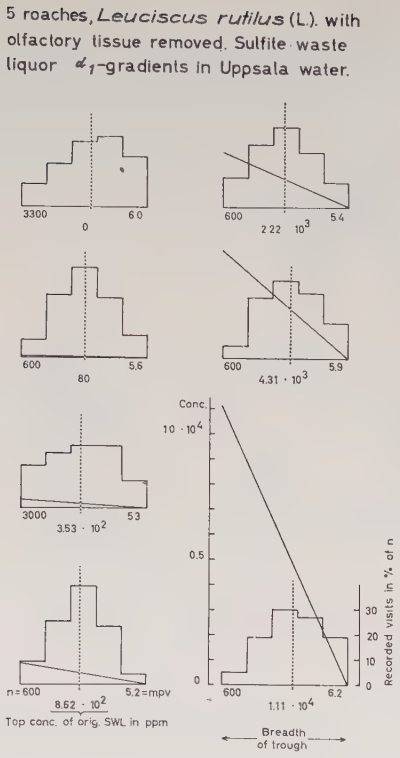


Fig. 46.

tions deriving from the different concentration steps of essentillay steeper gradients seems not to be improbable. These problems and possible quantitative relationships between stimulus and response with regard to the steepness of SWL gradients (cf., e.g. Weber-Fechner's law) have not yet been studied in any more detail.

In order to test the reliability of the interpretation presented here, rv arrived at in the steepest gradients of Figs. 42 and 44 have been compared with those given in pure pH/P_{CO_2} gradients of the same strength (Figs. 36 and 37). This comparison is shown in Table 16.

It appears from the survey in Table 16 that a good deal of the total avoidance given in the actual SWL gradient obtained in Uppsala water must be the result of olfactory stimulation. In the case of the same SWL gradient established in Hölle water the induced pH/P_{CO_2} gradient is steep enough to give rise to the rv arrived at in the actual SWL gradient. This does not exclude the possibility, however, that the roaches are also aware of the existing SWL gradients on account of olfactory perception.

To summarize the information arrived at in the present SWL experiments with the roach we return to Fig. 42. A rectilinear relationship between rv

Table 16. Reaction values (rv) arrived at with intact roaches in SWL gradients and pure pH/P_{CO_2} gradients with the same pH on the top concentration side.

	Uppsala water	Hölle water
pH on the top concentration side (10,000 — 15,000 ppm SWL).....	6.7	4.3
Rv in SWL gradients.....	— 3.0	— 2.9
Rv in pH/P_{CO_2} gradients	— 1.5	— 2.9

and the logarithm of the steepness (top concentration) of α_1-gradient of SWL seems to exist from a threshold value of about 0.2 ppm up to a top concentration of about 1000 ppm (cf. the statistics of the regression lines No. 1 and No. 2 in the figure). In steeper gradients additional effects of pH and CO_2 interfere with the rv obtained. This interference has not been studied in detail. The discriminating capacity against the odorous substances acting as directive stimuli diminish with increasing steepness in the steepest gradients (with higher top concentrations than about 6000 ppm). Thus apart from the effects of pH and CO_2 it must be assumed that the toxic effect of SWL *per se* increases at the same time as the ability to avoid high concentrations evidently diminishes. No connection between the ability to avoid and the toxic action of SWL can therefore be traced amongst roaches and minnows. This result is essentially the reverse of those arrived at in the experiments with pH and P_{CO_2}. In the latter cases the fish were able to avoid contacts with higher concentrations as long as tolerable or more favourable conditions were present (cf. p. 103).

3. *Other species*

The clear tendency in the steepest gradients towards regressive rv with increasing steepness of gradients is found only in the tests performed with *Leuciscus rutilus* and *Phoxinus phoxinus* (Figs. 39—42, and 44). In the cases of other species sufficiently steep gradients for a similar marked regression of the reaction curves (Figs. 47, 48, and 50) have, probably not been established. The typical none-or-all response displayed by salmonids and in a way also by perch fry (cf. Chapter IV) may also contribute to the progressively ascending shapes with increasing steepness of the reaction curves shown in Figs. 48 and 50.

In all cases, except those in Figs. 43, 49, and 51, weaker gradients, in which no reactions were obtained, are excluded from the graphical presentations. The reaction curve of Fig. 51 shows that intact specimens of *Gasterosteus aculeatus* do not exhibit any avoidance reactions at all, even in rather steep SWL gradients, that is, in those with a top concentration up to 2000 ppm. At this dilution the actual SWL (tapped in June, 1958; cf. Table 7)

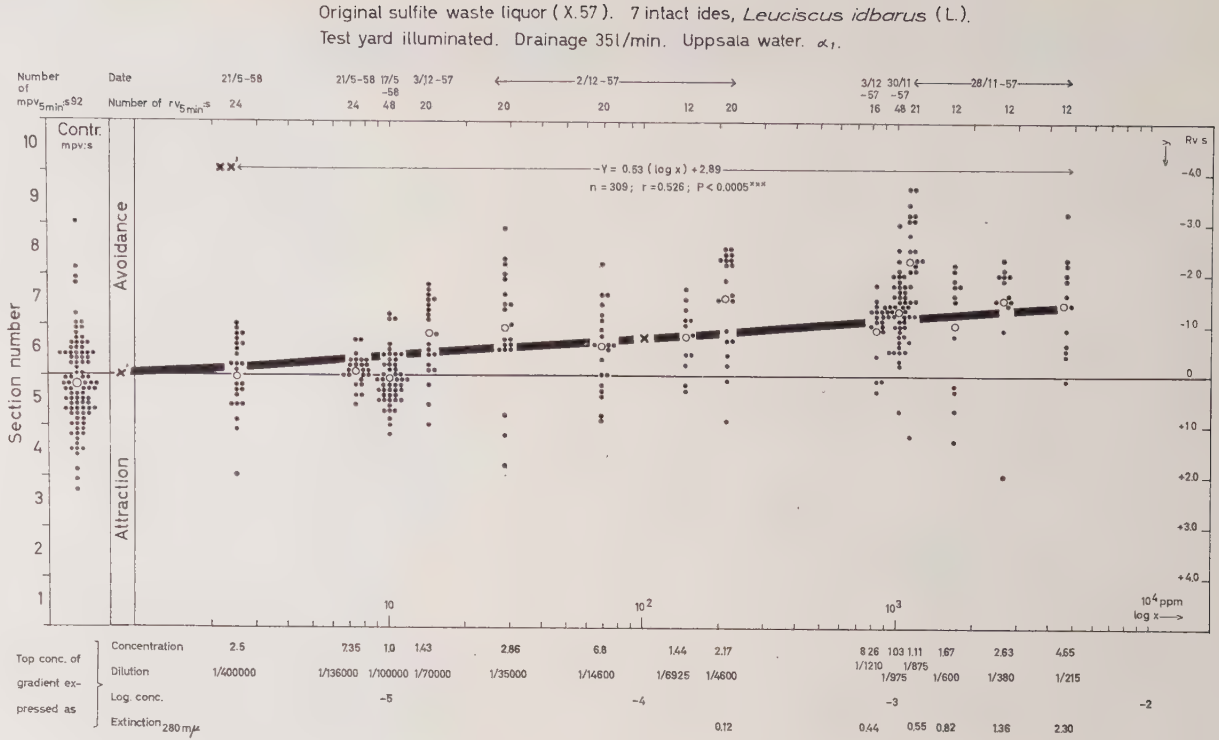


Fig. 47. Reaction diagram (cf. p. 56 and Fig. 42) showing the preference reactions of ides in graded environments of SWL. This species was tested at a flow rate of about 2 cm/sec. The regression line calculated on all rv_{5min} is shown, the position of the centre coordinates is shown at ×, the intersection with the median line at ×'.

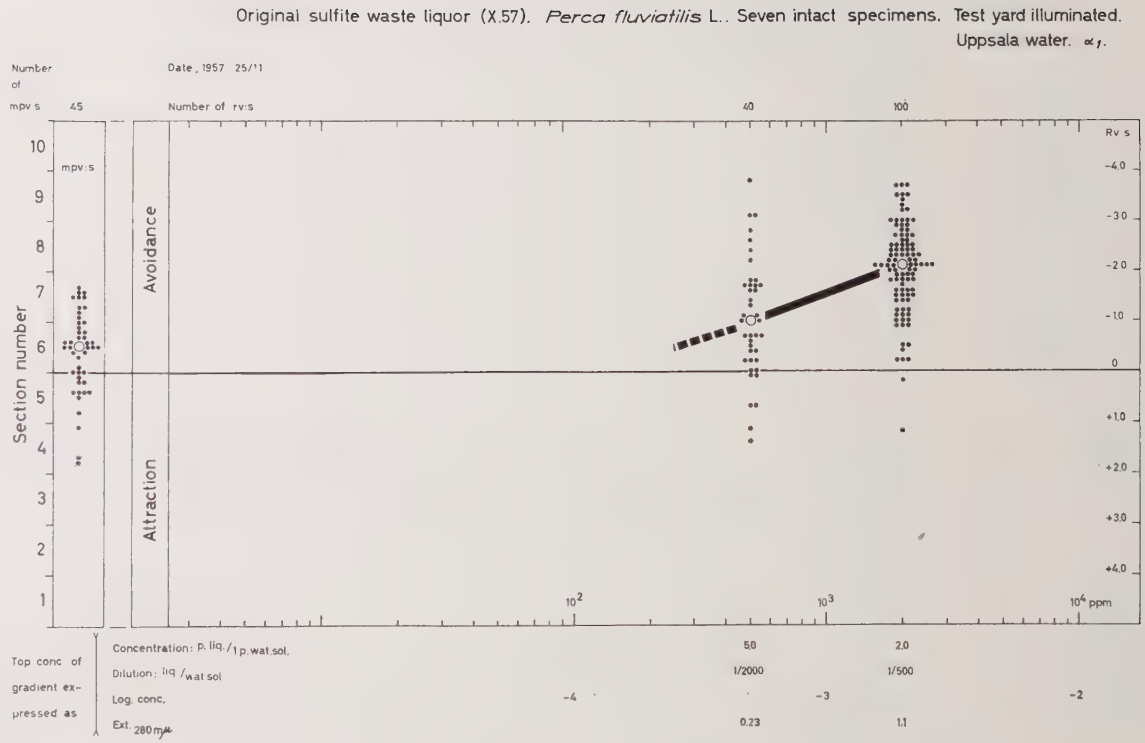


Fig. 48. Reaction diagram (cf. p. 56 and Fig. 42) based on the observational material also presented in Fig. 18.

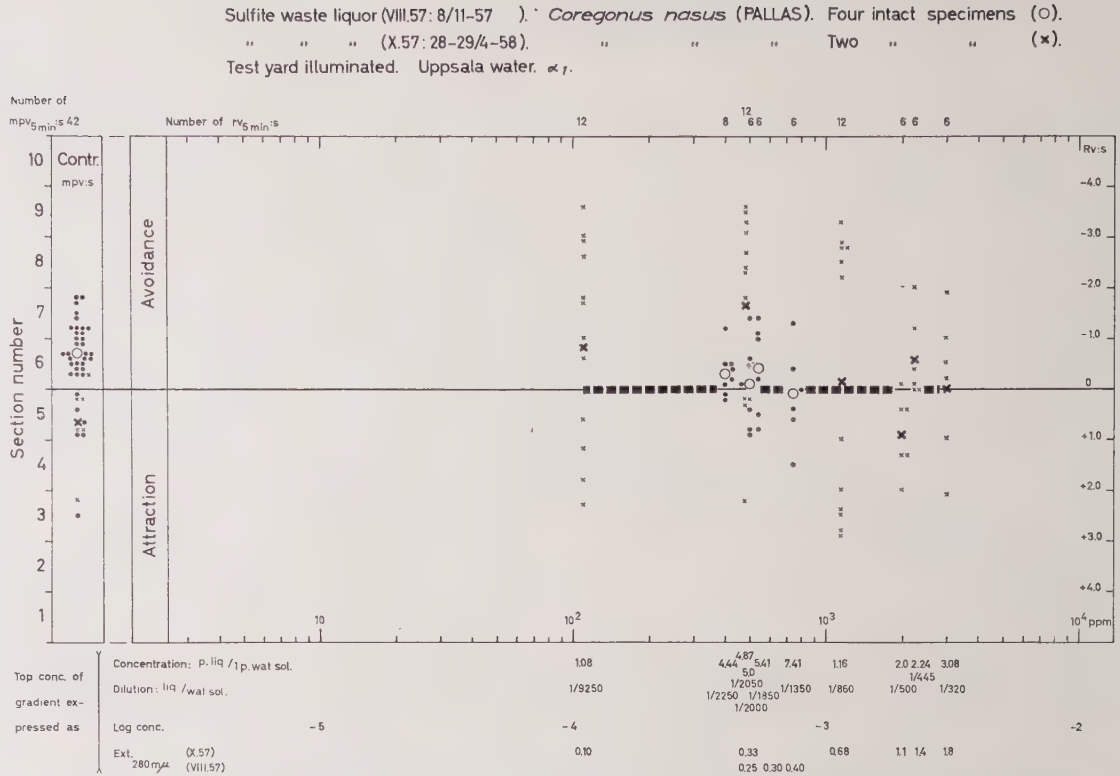

Sulfite waste liquor (VIII.57: 8/11–57). *Coregonus nasus* (PALLAS). Four intact specimens (○).
" " " (X.57: 28–29/4–58). " " " Two " " (×).
Test yard illuminated. Uppsala water. ∝ 1.
Number of mpv 5 min:s 42
Number of rv 5 min:s
Contr. mpv:s
Avoidance
Attraction
Section number
Rv:s
ppm
Top conc. of gradient expressed as
Concentration: p. liq / 1 p. wat sol.
Dilution: liq / wat sol.
Log conc.
Ext. 280 mμ (X.57) (VIII.57)

Fig. 49. Reaction diagram (cf. p. 56 and Fig. 42) showing the results of two experimental series performed with *Coregonus nasus*.

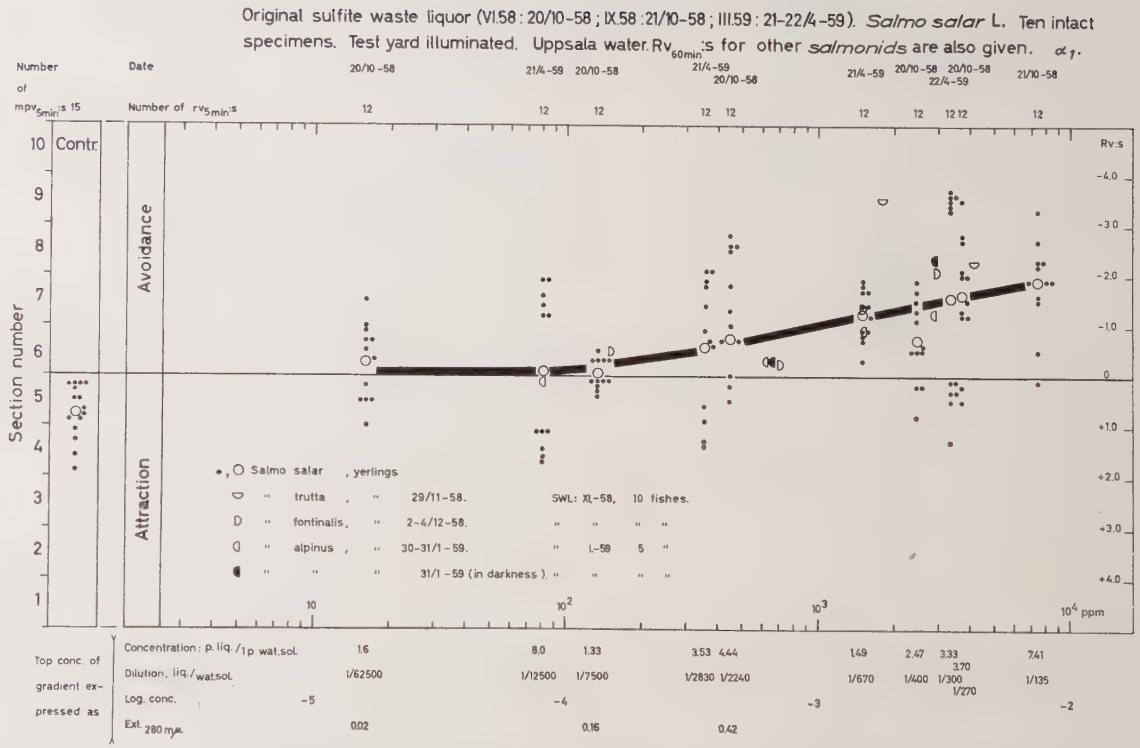

Original sulfite waste liquor (VI.58 : 20/10–58 ; IX.58 : 21/10–58 ; III.59 : 21–22/4–59). Salmo salar L. Ten intact specimens. Test yard illuminated. Uppsala water. Rv60min:s for other salmonids are also given. α1.
Number of mpv5min:s 15
Date
Number of rv5min:s
Section number
Contr.
Avoidance
Attraction
Rv:s
ppm
•, O Salmo salar, yerlings
d " trutta, " 29/11–58. SWL: XI–58, 10 fishes.
D " fontinalis, " 2–4/12–58. " " " "
G " alpinus, " 30–31/1–59. " I.–58 5 "
◖ " " " 31/1–59 (in darkness). " " " "
Top conc. of gradient expressed as
Concentration: p. liq. /1p wat.sol.
Dilution: liq./wat.sol.
Log. conc.
Ext. 280 mμ

Fig. 50. Reaction diagram (cf. p. 56 and Fig. 42) showing the preferences of various salmonid parr in SWL-gradients of different steepnesses.

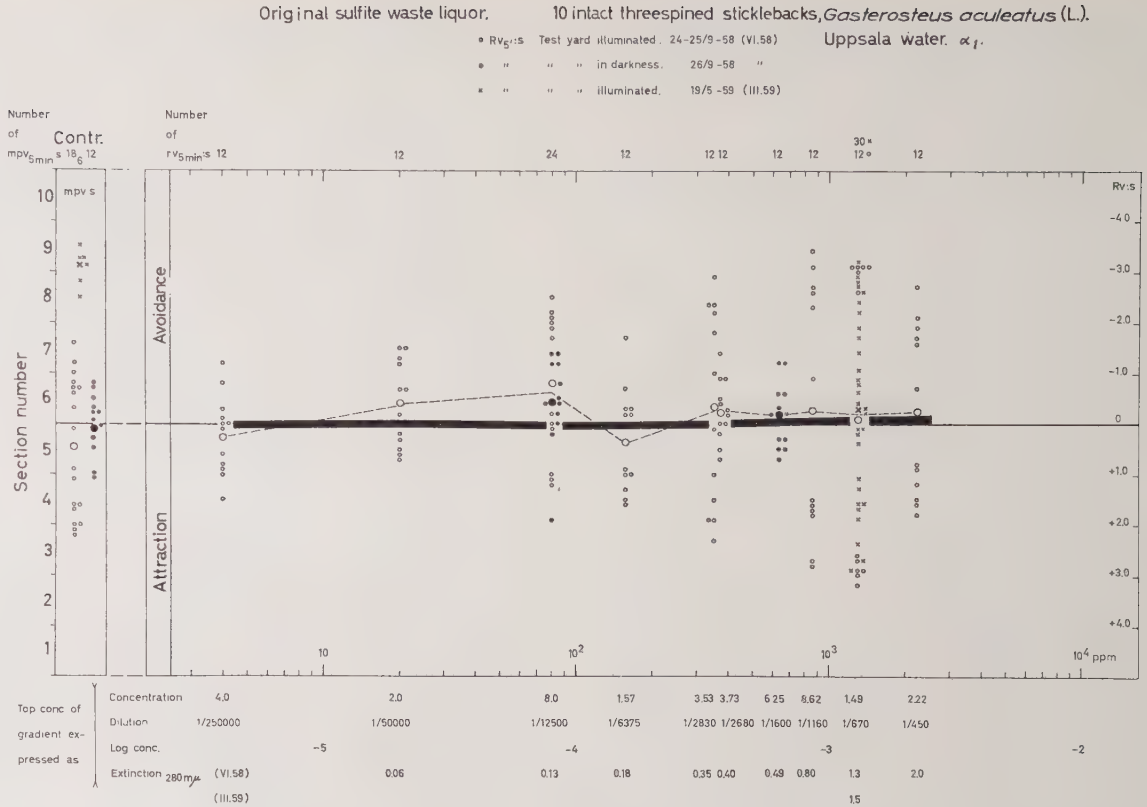

Original sulfite waste liquor. 10 intact threespined sticklebacks, Gasterosteus aculeatus (L.).
Rv5min:s Test yard illuminated. 24-25/9-58 (VI.58) Uppsala water. α1.
" " " in darkness. 26/9-58 "
" " " illuminated. 19/5-59 (III.59)
Number of mpv5min:s
Contr.
Number of rv5min:s
12 12 24 12 12 12 12 12 30 12 12
mpv:s
Rv:s
Avoidance
Attraction
Section number
10 9 8 7 6 5 4 3 2 1
-4.0 -3.0 -2.0 -1.0 0 +1.0 +2.0 +3.0 +4.0
10 10² 10³ 10⁴ ppm
Top conc of gradient expressed as
Concentration 4.0 2.0 8.0 1.57 3.53 3.73 6.25 8.62 1.49 2.22
Dilution 1/250000 1/50000 1/12500 1/6375 1/2830 1/2680 1/1600 1/1160 1/670 1/450
Log conc. -5 -4 -3 -2
Extinction 280mμ (VI.58) 0.06 0.13 0.18 0.35 0.40 0.49 0.80 1.3 2.0
(III.59) 1.5

Fig. 51. Reaction diagram (cf. p. 56 and Fig. 42) showing the non-existence of preference reaction due to α_1-gradients of SWL in experiments with three-spined sticklebacks. The symmetrical and comparatively wide dispersion of rv_{5min} on both sides of the median line is the result of the stationary behaviour of this species. A similar tendency can also be traced in Fig. 50.

has practically no influence upon pH and the carbonic acid protolytic system of the Uppsala water. As is seen in Fig. 49, this is also valid for the whitefish (*Coregonus nasus*) in still stronger gradients, that is, up to a top concentration of about 3000 ppm. Preliminary tests with pike fry (about six months old) indicated that this species do not show any preferences at all in steep α_1-gradients of SWL which were established in Uppsala water at a flow rate well beyond the usual 1 cm/sec. These results are in good agreement with the fact that both the stickleback and the pike have poorly developed olfactory senses (WUNDER, 1927, 1936; TEICHMANN, 1954).

Finally it must be emphasized that at the present stage of our information attraction and/or avoidance released by other senses than smell, as, for instance, by way of gustation, is not entirely out of the question. Of course, the possibility of the existence of counteractive and/or synergistic stimulations of different organs and by different substances contained in such a heterogenous test agent as SWL cannot be wholly disregarded. The non-existence of any pronounced preference reactions in the cases of roaches with their olfactory organs removed (Figs. 43 and 46), intact whitefish (Fig. 49), and intact sticklebacks (Fig. 51), however, does not argue in favour of the assumption that more pronounced reactions really occur, besides those considered here.

4. *A comparison of the reactions shown by different species*

The preference thresholds (p. 57) obtained for different species tested in the present α_1-gradient experiments with SWL are surveyed in Table 17. The top concentration of the gradient in which descending avoidance is first noticeable in the steepest gradients (cf. the right-hand parts of the reaction

Table 17. Schedule of critical SWL concentrations in the preference reactions of various fish as judged from the present experimental series with steeper and steeper α_1-gradients (cf. Figs. 39—51). The figures refer to ppm of original SWL mixed with Uppsala water.

Species	Approximate preference threshold	Top concentration of gradient with incipient descending avoidance reaction
Phoxinus phoxinus	1—10	2000—3000
Leuciscus rutilus	0.1—1	6000—7000
L. idbarus	0.1	—
Perca fluviatilus	< 100	—
Coregonus nasus	> 3000	—
Salmo salar, parr	100	—
S. trutta, parr	100	—
S. fontinalis, parr	100	—
S. alpinus, parr	100	—
Gasterosteus aculeatus ..	> 3000	—

curves of Figs. 39—42) is also presented for the minnow and the roach which are the only species studied in this respect.

A clear connection exists among various fish between the preference thresholds (Table 17), the significance of the olfactory sense in the search for food (WUNDER, 1927, 1936), and the extension of the olfactory epithelium (TEICHMANN, 1954). These facts support the statement made on p. 115 that avoidance in SWL gradients is greatly due to olfactory responses.

No correlation seems to exist, on the other hand, between the preference thresholds obtained and the toxicity of SWL.

Great discrepancies characterize the estimates of the tolerance limits which have been reported. Inevitable survival tests or not either easy to perform with such a heterogenous substance as SWL. In an extensive study WILLIAMS, ELRIDGE, MAINS, and LASATER (1953) determined the mortality "threshold indices" (approximately 5 % kill) for yearlings of three species belonging to the Genus *Oncorhynchus*. In long term experiments (30 days) and in the presence of sufficient oxygen (above 5 ppm) the critical concentration in sea water varied between 500 and 1500 ppm of original SWL which was standardized to 10 % solids. An approximate toxic limit of the same dimension or about 1000 ppm has also been stated for different species by other investigators (cf. KLASON, 1910; HOFER, 1914; PRAVDIN and KLIMOJA, 1939; PAWLINOWA, 1952; MOSSEWITSCH and GUSSEW, 1958). But, according to NIGHTINGALE and LOOSANOFF (1928) and NIGHTINGALE (1938) the exposition to 5000 ppm during 28 days have no effects upon salmon fry (cf. COLE, 1935, 1941). VALLIN (1935) reports that *Leuciscus rutilus* (about 5 cm) died after 30 hours and *Salmo salar* fry showed pronounced distress after 42 hours when exposed to SWL (10,000 ppm, pH 5.1, 9—10°C). Both species survived 90 minutes in 20,000 ppm (pH 4.0), but were unaffected after 48 hours in 5000 ppm (pH 6.2). After neutralization with NaOH they died within 40 hours at 20,000 ppm, but were not affected in 10,000 ppm and more diluted solutions. Also HALME (1950) claims that the toxicity of SWL is mainly due to pH. The direct causes of the toxicity of SWL to fish is not exactly known, however. The effects of pH, sulphureous compounds, resinous substances, and furfural have been suggested (MOSSEWITSCH and GUSSEW, 1958). Cf. also EBELING (1931, 1932).

It appears from Table 17 and Figs. 39—42 that minnows and roaches show gradually decreasing avoidance reactions in steeper gradients than those with the top concentrations of about 2000 ppm and 6000 ppm, respectively. At a comparison with the toxic limits quoted above, it is apparent that the avoidance reactions which are normally shown by these species in moderately steep SWL gradients, are disturbed by toxic influences from the highest SWL concentrations in the steepest ones. The readiness to avoid CO_2 is possibly also affected in the latter gradients (cf. pp. 115 and 119).

It must be concluded, at last, that the minnow and the roach are not able

to avoid high (toxic) concentrations of SWL though lower (less toxic or not toxic) concentrations are distinctly avoided after olfactory perception. Such a reaction pattern cannot be favourable for the survival of fish in SWL polluted areas.

5. *Final discussion*

More or less catastrophic declines in the catches of salmon and other fish have repeatedly been reported in connection with SWL pollution (Turing, 1952; Williams *et al.*, 1953; Smith and Saalfeld, 1955; and others). Unpublished observations during the last decades on the variation in the upstream migration of trout and salmon into some rivers in northern Sweden (Rickleån, Nätraån) can also be connected with the discharge of SWL (G. Öquist and S. Berg, oral communications). Pravdin and Klimoja (1939) report that salmonids, bream, eel, and roach are more sensitive to SWL than perch, pope, and pike, and that burbot and stickleback are still more resistant. Vallin (1935) submerged fish in cages at different distances from the outfalls of pulp mills. Salmonids and coregonids were found to be more sensitive than cyprinids, pike, and perch. Net-fishing at the same time also indicated a fairly dense occurrence of the last-mentioned species in rather severely SWL contaminated waters, while the first-mentioned ones were quite missing. According to Järnefelt (1940) SWL pollution in a Finnish lake resulted in a decline of the stock of whitefish (*Coregonus spp.*), while bream and pike-perch increased in number. Mossewitsch and Gussew (1958) report a decrease of certain species including the pike-perch in advance of roach and pike on account of pulp mill effluents. The present author has observed during the summers 1958 and 1959 a great abundance of roach, bleak, perch, and eel quite close to the drains from Svanö sulphite pulp mill.

It appears, maybe, from these scanty field observations, which are partly contradictory, that salmonids and coregonids are more sensitive to SWL pollution than other species. They also seem to disappear earlier from SWL polluted areas. Cyprinids, perch, and pike seem to be more resistant and to stay longer in environments with discharged SWL. Field observations usually do not distinguish the effects of depleted oxygen and/or other changes in the environmental factors which are induced by SWL. It appears from the present results that the guiding influence of SWL due to olfactory responses is not sufficient to explain the disappearing of fish from SWL polluted areas. Certainly a great complexity of incitements, not less by factors interacting with the respiratory processes, are involved. In most extreme cases SWL pollution brings about anaerobic conditions and the formation of H_2S. Such environments cannot be tolerated at all by higher aquatic organisms (cf. e.g. Vallin, 1955 a, 1939; Gustafsson, 1948) and seem to be avoided by fish (cf. p. 95; Shelford and Powers, 1915).

IV. General discussion

To sum up the results arrived at in the present study, some brief suggestions will finally be made on the biological significance of the preference reactions demonstrated. This will be done with some hypothetical applications to environmental conditions which may be encountered by fish in unaffected or polluted waters. O_2, pH, and CO_2 occur in different proportions in the natural habitats of fish and interfere greatly with the respiratory functions. Physiological adaptations to various O_2, (pH), and CO_2 regimes is accordingly evolved among aquatic animals, not less within Pisces (Carter and Beadle, 1930, 1931; Leiner, 1938; Krogh, 1941; Black, 1951; articles in Brown, 1957; and elsewhere). There is no reason to expect any corresponding physiological adaptations to SWL which is recently introduced by man as a new ecological factor in the original habitats of fish. O_2, pH, and CO_2 are here called "natural ecological factors" and SWL an "artificial ecological factor" (cf. p. 5).

The factors belonging to the first-mentioned group which are studied in the present fluviarium experiments act all at least partly as releasing stimuli (*sensu* Tinbergen, 1955). O_2 depletion, as well as raised CO_2 tension in combination with lowered pH, brings about a more or less intense, increased swimming activity (e.g. Figs 27 and 38). This is interpreted here as an appetitive behaviour or an emergency reaction which is presumably released by discharges from the central nervous system. The respiratory centre of the medulla or sympathetic elements of the autonomous system may be involved. As judged from the present results especially in the case of CO_2 possibly also reflexes upon external stimulation of CO_2 sensible chemoreceptors located in the gill region may be embraced. Accordingly, due to orthokinesis (*sensu* Fraenkel and Gunn, 1940), the test fishes will ultimately gather in the parts of the gradients where they are least active. Orthokinesis is exclusively responsible for the positive preference reactions (p. 49) which are shown in O_2 gradients. In pH/P_{CO_2} and SWL gradients on the other hand directive avoidance reactions dominate, in the last type almost completely and coupled with olfactory sensations.

Thus, of the "natural ecological factors" studied here, CO_2 and to some extent also pH, but not O_2, is found to be directive stimuli (*sensu* Tinbergen, 1955). But increased, random mobility also plays a rôle during the first few minutes of an experiment with combined pH/P_{CO_2} gradients. Then the probality is raised that the gradient is detected and the readiness of the fish to respond on the concentration differences is possibly also increased. When the fish swim against such a gradient (but not in a steep O_2 gradient) hesitation and rebound reactions are easily recognized at the confrontations with the concentration steps. The erratic swimming (appetitive) behaviour is thus coupled with directive stimulation which in juvenile salmonids leads to the

taking of new resting positions (consummatory action) on the bottom of the pure side of the test yard (Fig. 38). The latter response apparently takes place upon lack of further excitation and is possibly connected with an induced oxygen debt. Anyhow, all species studied are able with great accuracy to give the appropriate reactions which save them from a too long stay in the noxious concentrations of pH/P_{CO_2} gradients. That is, as long as areas with tolerable conditions were not missing, with very few exceptions all fish survived in the present pH/P_{CO_2} experiments.

In natural waters O_2 and CO_2 usually occur in mutually reversed quantities. Photosynthesis, respiration, and decaying processes contribute to this. Furthermore, in well aerated waters O_2, if lost, continually invades from the air while excess CO_2 evades from the water. In stagnant waters with fluorishing vegetation there are great fluctuations as O_2 is consumed and CO_2 increases during the night and *vice versa* in daylight. On account of the existance of simultaneous, but reversed O_2 and CO_2 gradients, areas with lack of oxygen in nature are probably in many casse avoided by fish through reactions which are constituted of two elements. (1) Orthokinetical random movements are initially released by lack of O_2 and increased CO_2 tension. (2) The critical areas are avoided by directive avoidance exclusively to CO_2. This way of looking at the matter was put forward by SHELFORD and ALLEE (1913). "Increased carbon dioxide is usually accompanied in nature by low oxygen and it is to the combination of lack of oxygen . . . and increased carbon dioxide, that the fish react most definitely" (*op. cit.*, p. 261). Both O_2 and CO_2 have since been repeatedly suggested to operate in the distribution of fish (JONES, 1952; POWERS, 1939, 1941, 1943, and elsewhere; FRY, 1947; BLACK, FRY, and BLACK, 1954; and others). Even if the statement quoted from SHELFORD and ALLEE (1913) is not verified in all details by fluviarium experiments, it seems reasonable to say at the present stage of information, that the combination of the actual factors may initiate migration and that CO_2 gradients may act as guideposts in the wanderings of fish within and between water basins and streams. The fish are there encountered with different strata, as for example in stratified lakes, within and near the vegetation belts (LINDROTH, 1942; PUKE, 1948). Gradients and more or less sudden changes in the respiratory gas content may also occur in the external medium at dawn and nightfall, at different meteorological conditions, at epilimnic circulation, and so on.

DOUDOROFF (1938) noticed that the greenfish, *Girella nigricans*, when tested in artificial temperature gradients did not select the temperatures in which it normally stays in nature. The present pH/P_{CO_2} gradient experiments indicate clearly a correlation between physiological adaptation to a certain respiratory gas regime in the natural habitat and the strength of avoidance to CO_2 (Fig. 36). It seems probable that the ability to avoid CO_2 and low pH has been evolved parallel to the physiological and ecological adaptations arisen during past ages of speciation (LACK, 1940).

The principle outlined here will not necessarily hold good in polluted areas because of at least two reasons. (1) The mutually reversed relationship between the contents of CO_2 and O_2 may be disturbed or extinguished. (2) The presence of one or more "artificial ecological factors" may act as a kind of masking factors (Fry, 1947). These may make it impossible for the fish to descry the original guiding influences from the environment and give the normal, appropriate reactions.

It is also apparent from the experiments performed with roach, minnow, and other species in SWL gradients that fish are not adapted to meet this "artificial ecological factor" with the appropriate responses which would be advantageous for individuals which are compelled to live along with such a factor in a recently polluted area. If the fish do not succumb or are driven out they may suffer from decreased vitality in the new environment.

To summarize once more: the biological significance of the abilities of free-moving species like fish to find their way out from and avoid adverse conditions in a graded environment must be emphasized (cf. Woodbury, 1956, Woker and Wuhrman, 1958). Responses which facilitate the functions necessary for life (or reproduction) are advantageous to the species. Most probably such responses have been evolved by means of natural selection vis-à-vis certain ecological factors occurring in the natural habitats of fish, such as O_2, pH, and/or CO_2, but less probably vis-à-vis many toxic or not toxic agents which are recently discharged into the waters from industrial and municipal plants, for instance SWL. In other words, the aquatic animals have been adapted to withstand the first kind of environmental factors only. It is therefore an important task to survey to what extent discharged matter forms barriers for migrating species and restricts the distribution of more stationary ones.

Possible guiding influences from environmental gradients may certainly not be imagined in each case as a simple positive or negative taxis along the road of migration. More probably, directional orientation is restricted to the nearest environment of moving fish, as for instance in the contact zones between different water masses. This does not necessarily mean that the fish move along, constantly following such bordering zones by perceiving different stimulations at sheers into one or the other water mass of different quality (the sheer or edge effect according to Hasler, 1956, p. 207). It seems more probably that migrating fish respond on one hand to differences in gas content, physical cues, or odours in the contact zone. These constitute signals for directional orientation which results in the fishes' remaining in one water mass. Within this they may respond actively by directional orientation to other stimuli as for instance current or are transported more passively (Hoar, 1954, 1956, 1958; Kalleberg, 1958, and oral communication).

Summary

The ability of aquatic animals to discriminate between different qualities in the surrounding medium can be studied with the fluviarium technique (HÖGLUND, 1953, 1960; LINDAHL and MARCSTRÖM, 1958). Using this technique the reactions of small specimens of fish (cf. Table 1, p. 12) to environmental differences in O_2, pH, CO_2, and SWL (sulphite waste liquor) have been studied comparatively in chemically well-defined, stable, and reproducible concentration gradients of different types and angles (Fig. 1).

The fish are allowed to swim freely about in a test yard which is a confined space (23.5×33 cm) of a streaming aquarium called a fluviarium. Perpendicularly to the direction of flow (1 cm/sec) ten uniformly wide (3.3 cm) concentration steps are established in different standard conditions. Gradients of various steepnesses all rising from nil at one side to a certain top concentration along the other are most frequently used. In the steepest gradients used in a series of experiments the test fish encountered concentrations well beyond the actual tolerable limits, at least in the most contaminated parts of the yard. Intact animals as well as fish with sense organs eliminated have been tested for at least 30 minutes in each standard gradient. The visits to each concentration were recorded at even intervals (usually every 30 seconds) with the aid of a film camera. The strength of preference reaction (avoidance or attraction) was measured as the lateral displacement (*rv*) from the median line of the test yard of the mean position value (*mpv*) of the distribution of records over the ten concentration steps, numbered 1 to 10. *Rv* and *mpv* are expressed in section number as unit (3.3 cm, *i.e.* one tenth of the width of the experimental trough; cf. above). The quantitative relationship between preference reaction and steepness of gradients is presented graphically *i.a.* in the form of 23 *reaction curves*. These were found to be characteristic of species and agents tested. They also indicate the actual *preference threshold values*.

The essential features of the innate behaviour shown in nature are recognized in the fluviarium. The fish material used in the experiments represents different physiological, ecological, and ethological types. The courses of the reaction curves of different species must be estimated against the specific behaviour displayed in the test yard under control conditions (Fig. 24). That is, when pure water is poured through the apparatus, some

cyprinids as for example the roach (*Leuciscus rutilus* L.) continually swim about in a rather aimless way. They steer up against the current (Fig. 20) and avoid to come into bodily contacts with the walls and the bottom of the test yard. When exposed to gradients of an environmental factor which acts as a directive stimulus, the animals hesitate or change direction mainly when meeting rising concentrations. Parr stages of the Atlantic salmon (*Salmo salar* L.), on the other hand, exhibit a typically stationary behaviour. When confronted with pH/P_{CO_2} gradients a more or less erratic free-swimming (appetitive) behaviour is released. This is sooner or later followed by a consummatory action, that is, seemingly upon the lack of further stimulation the parr take new resting positions on the bottom of the pure side of the test yard. Possible physiological mechanisms underlying these reactions have been discussed (Fig. 38).

The test agents were chosen as representatives of environmental factors which occur in all natural waters (O_2, pH, CO_2) and those which are newly introduced in the original habitats of fish (SWL). With respect to the division of abiotic, ecological factors into "natural" and "artificial" ones, the biological significance of response which makes it possible for free-moving aquatic animals to protect themselves against adverse influences from restricted parts of environmental gradients was finally considered.

The main results and conclusions arrived at as regards particular factors can be summarized in the following way.

(A) *Oxygen*

(1) In oxygen gradients rising from less than 1 mg/l, roach, salmon parr, and crayfish prefer to stay in the higher concentrations.

(2) The intensity of preference reaction is due to (*a*) the specific behaviour in the test yard and (*b*) the critical oxygen tension for particular species.

(3) Oxygen is a non-directive stimulus to fish and crayfish.

(4) Oxygen deficiency releases an emergency reaction which is characterized, inter alia by increased swimming activity. This may be regarded as an appetitive behaviour.

(5) The positive reactions (cf. p. 49) obtained in pure oxygen gradients are due to ortho-kinesis.

(B) *Acidity and carbon dioxide*

The main conclusions arrived at in the section concerning the reactions studied in combined pH/P_{CO_2} gradients can be summarized as stated below.

(1) All species studied in the present experiments are able to avoid adverse conditions in pH and combined pH/P_{CO_2} gradients.

(2) Fish are able to detect and avoid CO_2 separately from the accompanying pH.

(3) Fish show marked avoidance to molecularly dissolved CO_2.

(4) Roaches show avoidance of lower pH than about 5.6 and Atlantic salmon parr to lower pH than about 5.3.

(5) At the concentrations existing in the present experiments HCO_3^-, Na^+, and Cl^- are non-directive factors in the reactions of the roach.

(6) Hydrogen ions are non-directive upon the reactions of roach within the pH range of c. 5.6—10.5, and to the salmon parr from pH 5.3 to at least 7.4.

(7) Concerning pH, a certain correlation seems to exist between the tolerance limits and the directive influence upon fish.

(8) Regarding various species, a certain connection is found between the avoidance reactions to CO_2 and the narcotic effects of CO_2. No corresponding connection is found as regards pH.

(9) Compared with roach, salmon parr give sharper avoidance reactions to CO_2, but less pronounced avoidance to pH.

(10) The removal of olfactory tissues and the sectioning of the nerves innervating the lateral organs do not essentially change the reactions of roach, minnow, and salmon parr in pH/P_{CO_2} gradients.

(11) Acidity and carbon dioxide are either perceived by different receptor systems or by the same receptors at essentially different thresholds.

(12) The possibility is discussed that the avoidance reactions in CO_2 gradients may be attributed to a special CO_2 sense connected with chemoreceptors in the gill region.

(C) *Sulphite waste liquor (SWL)*

(1) Avoidance reactions in SWL gradients are to a great extent due to olfactory sensations. The removal of the olfactory tissues of minnows and roaches essentially extinguishes the avoidance shown by intact specimens especially in the lower concentration ranges of the reaction curves (Table 15, p. 114; Figs. 42, 43, and 45—46). A good correlation is found between the development of the sense of smell among various species and the preference thresh-old values obtained in SWL gradients (Table 17, p. 128).

(2) The amount of avoidance due to odorous substances contained in the SWL diminishes in the steepest gradients. Thus no connection is obtained between the presumed incipient detrimental or lethal limits on one hand and the ability of avoiding contacts with noxious concentrations on the other. On the contrary, the sensibility [1] of the olfactory receptors or the responsiveness on the whole to sensory stimuli evidently is depressed in the steepest gradients of SWL.

(3) On account of the free hydrogen ion content in SWL increasing avoidance due to free CO_2 which is liberated from the bicarbonate content is obtained in the steepest gradients established in hard, well-buffered waters (like Uppsala water) and to free hydrogen ions *per se* in steep SWL gradients

[1] Cf. the foot-note on p. 106.

in soft, poorly buffered waters (like Hölle water). The reactions to these additional factors presumably delay the descending slopes in the upper concentration ranges in the reaction curves of the minnow and the roach (Figs. 39—44).

(4) The discharge of SWL into the water basins may influence unfavourably upon fish life by at least two reasons. (a) In low, not toxic concentrations SWL may repel fish, especially those with well developed olfactory senses. (b) At high, toxic concentrations the ability of fish to avoid contacts with SWL diminishes.

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